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Review

From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites

Ara Koh,[1] Filipe De Vadder,[1] Petia Kovatcheva-Datchary,[1] and Fredrik Bäckhed[1,2,*]
[1]Wallenberg Laboratory and Sahlgrenska Center for Cardiovascular and Metabolic Research, Department of Molecular and Clinical Medicine, Institute of Medicine, University of Gothenburg, 413 45 Gothenburg, Sweden
[2]Novo Nordisk Foundation Center for Basic Metabolic Research, Section for Metabolic Receptology and Enteroendocrinology, Faculty of Health Sciences, University of Copenhagen, 2200 København, Denmark
*Correspondence: fredrik@wlab.gu.se
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A compelling set of links between the composition of the gut microbiota, the host diet, and host physiology has emerged. Do these links reflect cause-and-effect relationships, and what might be their mechanistic basis? A growing body of work implicates microbially produced metabolites as crucial executors of diet-based microbial influence on the host. Here, we will review data supporting the diverse functional roles carried out by a major class of bacterial metabolites, the short-chain fatty acids (SCFAs). SCFAs can directly activate G-coupled-receptors, inhibit histone deacetylases, and serve as energy substrates. They thus affect various physiological processes and may contribute to health and disease.

Introduction

The human microbiota is the collection of microbes that live on and in our body, with the largest and most diverse cluster of microorganisms inhabiting the gut. The gut microbiota has co-evolved with the host, which provides the microbes with a stable environment while the microbes provide the host with a broad range of functions such as digestion of complex dietary macronutrients, production of nutrients and vitamins, defense against pathogens, and maintenance of the immune system. Emerging data have demonstrated that an aberrant gut microbiota composition is associated with several diseases, including metabolic disorders and inflammatory bowel disorder (IBD). One of the mechanisms in which microbiota affects human health and disease is its capacity to produce either harmful metabolites associated with development of disease or beneficial metabolites that protect against disease. Diet drives gut microbiota composition and metabolism, making microbes a link between diet and different physiological states via their capacity to generate microbial metabolites depending on dietary intake. Some studies representing evidence of the interplay between diet, microbial composition, and physiology are described in the next paragraph, and the Review will then focus on a particularly versatile class of microbial metabolite short-chain fatty acids (SCFAs) that are derived from microbial fermentation of dietary fibers and are likely to have broad impacts on various aspects of host physiology.

Human populations with a diet enriched in complex carbohydrates, such as the Hadza hunter gatherers from Tanzania, have increased diversity of the gut microbiota (Schnorr et al., 2014). In contrast, long-term intake of high-fat and high-sucrose diet can lead to the extinction of several taxa of the gut microbiota (Sonnenburg et al., 2016). Barley kernel-based bread consumption improved glucose tolerance in healthy individuals with normal body mass index (BMI) in association with enrichment of *Prevotella copri* and increased capacity to ferment complex polysaccharides (Kovatcheva-Datchary et al., 2015). Improved postprandial glucose response and enrichment of butyrate-producing bacteria were found after 3 months intake of a mixture of inulin and oligofructose in obese women (Dewulf et al., 2013), and in mice that are obese due to either genetic manipulation or diet, supplementation with inulin-type fructans (fructo-oligosaccharides [FOS]) induced a remarkable increase of the number of *Bifidobacterium spp*, which is inversely correlated with adiposity and glucose intolerance (Cani et al., 2007).

Microbial Fermentation Products: Short-Chain Fatty Acids

Dietary fibers, but also proteins and peptides, which escape digestion by host enzymes in the upper gut, are metabolized by the microbiota in the cecum and colon (Macfarlane and Macfarlane, 2012). The major products from the microbial fermentative activity in the gut are SCFAs—in particular, acetate, propionate, and butyrate (Cummings et al., 1987). However, when fermentable fibers are in short supply, microbes switch to energetically less favorable sources for growth such as amino acids from dietary or endogenous proteins, or dietary fats (Cummings and Macfarlane, 1991; Wall et al., 2009), resulting in reduced fermentative activity of the microbiota and SCFAs as minor end products (Russell et al., 2011). Protein fermentation can contribute to the SCFA pool but mostly gives rise to branched-chain fatty acids such as isobutyrate, 2-methylbutyrate, and isovalerate, exclusively originating from branched-chain amino acids valine, isoleucine, and leucine (Smith and Macfarlane, 1997), which are implicated in insulin resistance (Newgard et al., 2009). Further supplementation of diet rich in protein or fat with dietary fiber

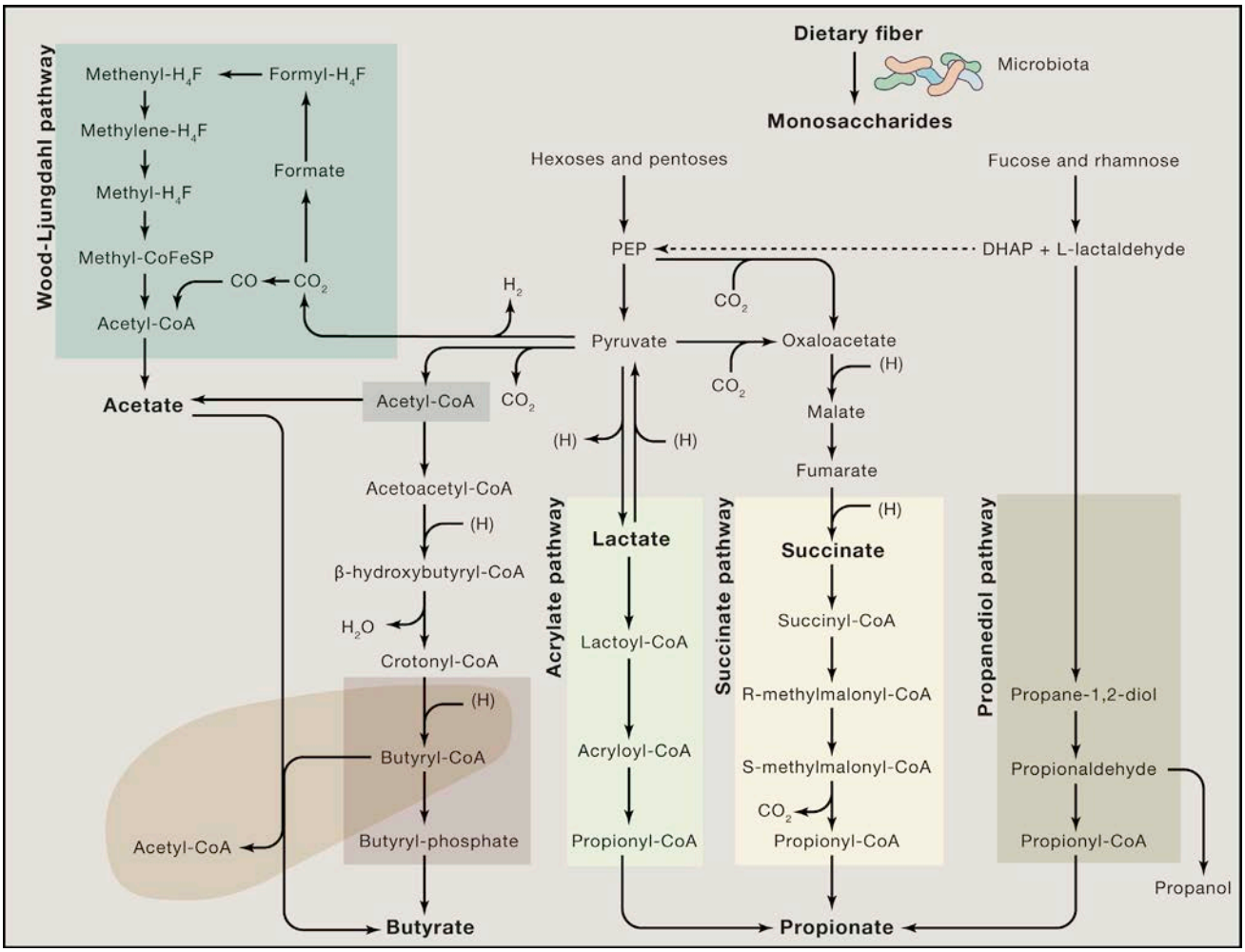

Figure 1. Known Pathways for Biosynthesis of SCFAs from Carbohydrate Fermentation and Bacterial Cross-Feeding

The microbial conversion of dietary fiber in the gut results in synthesis of the three major SCFAs, acetate, propionate, and butyrate. Acetate is produced from pyruvate via acetyl-CoA and also via the Wood-Ljungdahl pathway. Butyrate is synthesized from two molecules of acetyl-CoA, yielding acetoacetyl-CoA, which is further converted to butyryl-CoA via β-hydroxybutyryl-CoA and crotonyl-CoA. Propionate can be formed from PEP through the succinate pathway or the acrylate pathway, in which lactate is reduced to propionate. Microbes can also produce propionate through the propanediol pathway from deoxyhexose sugars, such as fucose and rhamnose. PEP, phosphoenolpyruvate; DHAP, dihydroxyacetonephosphate.

restores the levels of beneficial microbes, lowers the levels of toxic microbial metabolites, and increases SCFAs (Sanchez et al., 2009).

SCFA Biosynthesis, Absorption, and Distribution

The microbial conversions of dietary fiber to monosaccharides in the gut involve a number of principal events (reactions) mediated by the enzymatic repertoire of specific members of the gut microbiota (Figure 1 and Table 1). Major end products from these fermentations are the SCFAs. One of the major SCFAs, acetate, can be produced from pyruvate by many gut bacteria either via acetyl-CoA or via the Wood-Ljungdahl pathway in which acetate is synthesized via two branches: (1) the C_1-body branch (also known as Eastern branch) via reduction of CO_2 to formate and (2) the carbon monoxide branch (the Western branch) via reduction of CO_2 to CO, which is further combined with a methyl group to produce acetyl-CoA (Ragsdale and Pierce, 2008). Another major SCFA, propionate, is produced from succinate conversion to methylmalonyl-CoA via the succinate pathway. Propionate can also be synthesized from acrylate with lactate as a precursor through the acrylate pathway (Hetzel et al., 2003) and via the propanediol pathway, in which deoxyhexose sugars (such as fucose and rhamnose) are substrates (Scott et al., 2006). The third major SCFA, butyrate is formed from the condensation of two molecules of acetyl-CoA and subsequent reduction to butyryl-CoA, which can be converted to butyrate via the so-called classical pathway, by phosphotransbutyrylase and butyrate kinase (Louis et al., 2004). Butyryl-CoA can also be transformed to butyrate by the butyryl-CoA:acetate CoA-transferase route (Duncan et al., 2002). Some microbes in the gut can use both lactate and acetate to synthesize butyrate (Table 1), which prevents

Table 1. SCFA Production by Microbes in the Gut

SCFAs	Pathways/Reactions	Producers	References
Acetate	from pyruvate via acetyl-CoA	most of the enteric bacteria, e.g., *Akkermansia muciniphila*, *Bacteroides* spp., *Bifidobacterium* spp., *Prevotella* spp., *Ruminococcus* spp.	Louis et al., 2014; Rey et al., 2010
	Wood-Ljungdahl pathway	*Blautia hydrogenotrophica*, *Clostridium* spp., *Streptococcus* spp.	
Propionate	succinate pathway	*Bacteroides* spp., *Phascolarctobacterium succinatutens*, *Dialister* spp., *Veillonella* spp.	Louis et al., 2014; Scott et al., 2006
	acrylate pathway	*Megasphaera elsdenii*, *Coprococcus catus*	
	propanediol pathway	*Salmonella* spp., *Roseburia inulinivorans*, *Ruminococcus obeum*	
Butyrate	phosphotransbutyrylase/ butyrate kinase route	*Coprococcus comes*, *Coprococcus eutactus*	Duncan et al., 2002; Louis et al., 2014
	butyryl-CoA:acetate CoA-transferase route	*Anaerostipes* spp. (A, L), *Coprococcus catus* (A), *Eubacterium rectale* (A), *Eubacterium hallii* (A, L), *Faecalibacterium prausnitzii* (A), *Roseburia spp.* (A)	

A, acetate is the substrate for producing butyrate; L, lactate is the substrate for producing butyrate.

accumulation of lactate and stabilizes the intestinal environment. Analysis of metagenome data also suggested that butyrate can be synthesized from proteins via the lysine pathway (Vital et al., 2014), further suggesting that microbes in the gut can adapt to nutritional switches in order to maintain the synthesis of essential metabolites such as SCFAs.

The concentration of SCFAs varies along the length of the gut, with highest levels in the cecum and proximal colon, while it declines toward the distal colon (Cummings et al., 1987). Reduced SCFA concentrations may be explained by increased absorption through the Na^+-coupled monocarboxylate transporter SLC5A8 and the H^+-coupled low-affinity monocarboxylate transporter SLC16A1. Butyrate is the preferred energy source for colonocytes and is locally consumed, whereas other absorbed SCFAs drain into the portal vein. Propionate is metabolized in the liver and thus is only present at low concentration in the periphery, leaving acetate as the most abundant SCFA in peripheral circulation (Cummings et al., 1987) (Table 2). Furthermore, acetate can cross the blood-brain barrier and reduce appetite via a central homeostatic mechanism (Frost et al., 2014). Despite the low concentration in the periphery, propionate and butyrate affect peripheral organs indirectly by activation of hormonal and nervous systems. In the next sections, we discuss recent findings on microbially produced SCFAs and how they affect host physiology and pathology.

SCFAs as Signaling Molecules

HDAC Inhibitors

Histone acetylation emerges as a central switch that allows interconversion between permissive (via acetylation) and repressive chromatin structures (via deacetylation). Histone acetylation, which takes place on the epsilon amino groups of lysine residues on N-terminal tails of mainly histones 3 and 4, is thought to increase accessibility of the transcriptional machinery to promote gene transcription. Acetyl groups are added to histone tails by histone acetyltransferases (HATs) and are removed by histone deacetylases (HDACs). HDAC inhibitors have been widely used for cancer therapy. Their anti-inflammatory or immune-suppressive function has also been reported. Butyrate and, to a lesser extent, propionate are known to act as HDAC inhibitors (Johnstone, 2002); therefore, SCFAs may act as modulators of cancer and immune homeostasis.

Among the SCFAs, butyrate has been investigated most extensively. Present at high levels (mM) in the gut lumen, butyrate is the primary energy source for colonocytes and also protects against colorectal cancer and inflammation, at least partly by inhibiting HDACs (Flint et al., 2012), altering the expression of many genes with diverse functions, some of which include cell proliferation, apoptosis, and differentiation. In contrast to colorectal cancer cells, butyrate does not inhibit cell growth when it is delivered to healthy colonic epithelium in rodents or when it is added to noncancerous colonocytes in vitro. Instead, butyrate has either no significant effect or the opposite effect of stimulating cell growth under these conditions by acting as an energy substrate (Lupton, 2004)—the butyrate paradox. This may be explained by the fact that butyrate is the preferred energy substrate for normal colonocytes, whereas cancerous colonocytes prefer glucose (aerobic glycolysis or Warburg effect). Compared to normal colonocytes that oxidize butyrate, butyrate is accumulated 3-fold in nuclear extracts from cancer cells, generating higher concentrations of butyrate in cancerous epithelial cells, where it can act as an efficient HDAC inhibitor (Donohoe et al., 2012). Thus, butyrate may act as an HAT activator in normal cells and as an HDAC inhibitor in cancerous cells. The butyrate consumption of normal colonocytes protects stem/progenitor cells in the colon from exposure to high butyrate concentrations and alleviates butyrate-dependent HDAC inhibition and impairment of stem cell function (Kaiko et al., 2016). In contrast, butyrate-induced HDAC inhibition in small intestinal stem cells promotes the stem cell population (Yin et al., 2014). Taken together, the butyrate can induce different effects in a cell- and environment-specific context.

In addition to being an anti-tumor agent, SCFA-mediated HDAC inhibition is also a potent anti-inflammatory agent. Butyrate suppresses proinflammatory effectors in lamina

Table 2. Microbial Metabolites and Their Cognate Receptors

GPR43/FFAR2 (G_i, G_q)	Ligand	EC_{50}	Systemic/Portal Conc	References
	acetate (C2), propionate (C3)	259∼537 μM	70 μM /250 μM for C2; 5 μM /88 μM for C3	Brown et al., 2003; Kimura et al., 2013; Maslowski et al., 2009; Nøhr et al., 2013; Smith et al., 2013; Tolhurst et al., 2012
	Expression		Function	Microbial Metabolite-Mediated Signaling[a]
	colonic, small intestinal epithelium, EEC, colonic LP cells (mast cells, neutrophils, eosinophils, and colonic Tregs), leukocytes in small intestinal LP, polymorphonuclear cells, adipocytes, skeletal muscle, heart, and spleen		Metabolism: anti-lipolysis, increased insulin sensitivity and energy expenditure, GLP-1 and PYY secretion, preadipocyte differentiation, and appetite control; Cancer and IBD: protection against IBD, resolution of inflammation in animal models of colitis, and apoptosis of human colon cancer cell line; Immune: expansion and differentiation of Tregs, increase of Teff against pathogenic bacteria, neutrophil chemotaxis, reduced leukemia cell proliferation, and resolution of arthritis and asthma; Ect: electrolyte and fluid secretion	yes in intestinal epithelium and in LP cells; yes in adipocytes after consuming dietary fiber
GPR41/ FFAR3 (G_i)	Ligand	EC_{50}	Systemic/Portal Conc	References
	propionate (C3), butyrate (C4), (C3>C4>>C2)	12∼274 μM for C3	5 μM /88 μM for C3; 4 μM /29 μM for C4	Brown et al., 2003; De Vadder et al., 2014; Kimura et al., 2011; Le Poul et al., 2003; Nøhr et al., 2015; Samuel et al., 2008; Trompette et al., 2014
	Expression		Function	Microbial Metabolite-Mediated Signaling[a]
	colonic, small intestinal epithelium, colonic LP cells (mast cells but not in neutrophils), spleen, bone marrow, lymph nodes, adipose tissue, periportal afferent system, peripheral nervous system, peripheral blood monocuclear cells, pancreas, and co-expressed with GLP-1 in EECs located in the crypts and lower part of the villi		Metabolism: increased energy expenditure, oxygen consumption rate, leptin expression, decrease of food intake, increased PYY expression, and intestinal gluconeogenesis (IGN); Immune: hematopoiesis of DCs from bone marrow, increased Treg cells and DC precursors alleviating asthma, and protective immunity	yes in periportal afferent system, DC precursors in bone marrow, and intestinal epithelium
GPR109A/ HCA2 (G_i, $G_{\beta\gamma}$)	Ligand	EC_{50}	Systemic/Portal Conc	References
	niacin, β-D-OHB, butyrate (C4)	0.8 mM (h) and 0.3 mM (m) for β-D-OHB; 0.7 mM (h) and 1.6 mM (m) for butyrate	<0.1 μM for niacin; 1–2 mM (2–3 days of fasting) for β-D-OHB; 4 μM /29 μM for C4	Macia et al., 2015; Singh et al., 2014; Taggart et al., 2005; Thangaraju et al., 2009; Tunaru et al., 2003; Wise et al., 2003
	Expression		Function	Microbial Metabolite-Mediated Signaling[a]
	apical membrane of colonic/small intestinal epithelium (silenced in colon cancer and microbiota-dependent expression), macrophages, monocytes, neutrophils, DCs; but not in lymphocytes, adipocytes (white and brown), epidermal Langerhans cells, and retinal pigment epithelium		Metabolism: anti-lipolysis and triglyceride lowering; Cancer and IBD: protection against colitis and CRC, improved epithelial barrier function, and tumor suppressor in mammary gland; Immune: increase of Treg generation (FoxP3 expression), IL-10-producing T cells, and decrease of pro-inflammatory Th17 cells (only in colonic LP)	no evidence for niacin and β-D-OHB; yes in intestinal epithelium and DCs for butyrate

(*Continued on next page*)

Table 2. *Continued*

GPR81/HCA1 (G_i)	Ligand	EC_{50}	Systemic/Portal Conc	References
	lactate	5 mM (L-lactate), >20 mM (D-lactate)	3–5 mM (exercise), 10–50 mM (vaginal secretion)	Cai et al., 2008; Liu et al., 2009
	Expression		Function	Microbial Metabolite-Mediated Signaling[a]
	predominantly in adipose tissue (white and brown); minor in kidney, skeletal muscle, liver, rat brain (hippocampus, cerebellum; low level in the cortex, mostly in neurons, and less in astrocytes), human brain (pituitary gland), mouse primary cortical neuronal cells, intestinal tissue, and macrophages		Metabolism: anti-lipolysis, modulation of cortical neuron activity, and enterocyte turnover in response to starvation-refeeding; Cancer and IBD: reduced symptom in mouse models of hepatitis and pancreatitis; Immune: anti-inflammatory on macrophages (independent of G_i but dependent on β-arrestin2 signaling)	no evidence but maybe possible in vaginal tract
GPR91/SUCNR1 (G_i, G_q)	Ligand	EC_{50}	Systemic/Portal Conc	References
	succinate	56 μM (h), 28 μM (m)	2–3 μM (h), 6–20 μM (m), 1–3 mM (large intestine)	Ariza et al., 2012; Rubic et al., 2008
	Expression		Function	Microbial Metabolite-Mediated Signaling[a]
	WAT>kidney>trachea>dorsal root ganglia, liver, spleen, small intestine, quiescent hepatic stellate cells, heart, immature DCs, and retinal ganglion cell layer		Metabolism: activation of intrarenal renin-angiotensin system, hypertension, oxygen-induced retinopathy, decreased energy expenditure, impaired glucose tolerance, cardiac hypertrophy, and induction of VEGF and angiogenesis; Immune: activation of quiescent hepatic stellate cells in the ischemic liver and activation of DCs to augment immune response	no direct evidence

Abbreviations: EEC, enteroendocrine cell; LP, lamina propria; Tregs, regulatory T cells; GLP-1, glucagon like peptide-1; PYY, peptide YY; IBD, inflammatory bowel disease; Teff, effector T cell; DCs, dendritic cells; β-D-OHB, β-D-hydroxybutyrate; CRC, colorectal cancer; VEGF, vascular endothelial growth factor; microbial metabolite-mediated signaling[a], signaling through the receptors by microbially produced metabolites (not endogenously produced from the host).

propria macrophages (Chang et al., 2014) and differentiation of dendritic cells from bone marrow stem cells (Singh et al., 2010) via HDAC inhibition, making our immune system hyporesponsive to beneficial commensals. SCFAs also regulate cytokine expression in T cells and generation of regulatory T cells (Tregs) through HDAC inhibition. Effector T cells (Th1, Th2, and Th17 cells) have enhanced aerobic glycolysis, and inhibition of glycolysis promotes Treg cell generation (Shi et al., 2011). Thus, the metabolic shift in activated T cells will make them sensitive to SCFA-mediated HDAC inhibition, which may result in increased FoxP3 induction through acetylation at *FoxP3* locus (Arpaia et al., 2013; Furusawa et al., 2013). Interestingly, acetate—traditionally not regarded as an HDAC inhibitor—was found to inhibit HDACs in activated T cells (Park et al., 2015). Taken together, HDAC-inhibiting activity of SCFAs and concomitant beneficial health outcomes should be considered together with their production (mM range), transport (μM range), and energetics of cells (oxidative phosphorylation versus glycolysis).

Ligands for GPCRs

The human genome possesses ~800 GPCRs, and recently a cluster of four GPCR genes (named *GPR40* to *GPR43*) was identified in close proximity to the *CD22* gene on chromosome 19q13.1. These are also called free fatty acid receptors (FFARs) since they sense free fatty acids. In 2003, three independent research groups deorphanized GPR43 and GPR41 (Brown et al., 2003; Le Poul et al., 2003; Nilsson et al., 2003), which were renamed FFAR2 and FFAR3, respectively. Here, we focus on the distribution of SCFA receptors in relation to SCFA concentration and effective concentration toward its cognate receptors to discuss the relevance of SCFAs as signaling molecules (Table 2 and Figure 2).

GPR43/FFAR2 is a $G_{i/o}$- and G_q-dual-coupled GPCR, but recent studies have shown that its functions are mainly mediated by $G_{i/o}$ (Tolhurst et al., 2012). The one exception is the intestine, where GPR43 is G_q coupled, promoting GLP-1 secretion in L cells (Tolhurst et al., 2012). Acetate and propionate are the most potent activators of GPR43. The EC_{50} for acetate and propionate is ~250–500 μM (Le Poul et al., 2003). Acetate and propionate in the lumen of the colon range from 10 to 100 mM, and GPR43 is expressed in the colonic epithelial cells. Thus, GPR43 should continuously be saturated with ligands, and subtle variations in SCFA concentrations should not affect signaling. However, the colon has a very thick layer of mucus, continuous mucus flow, and peristalsis, which will induce a SCFA gradient (Donohoe et al., 2012), so the observed concentrations of acetate and propionate likely will be in a bioactive-relevant range for activating GPR43 in

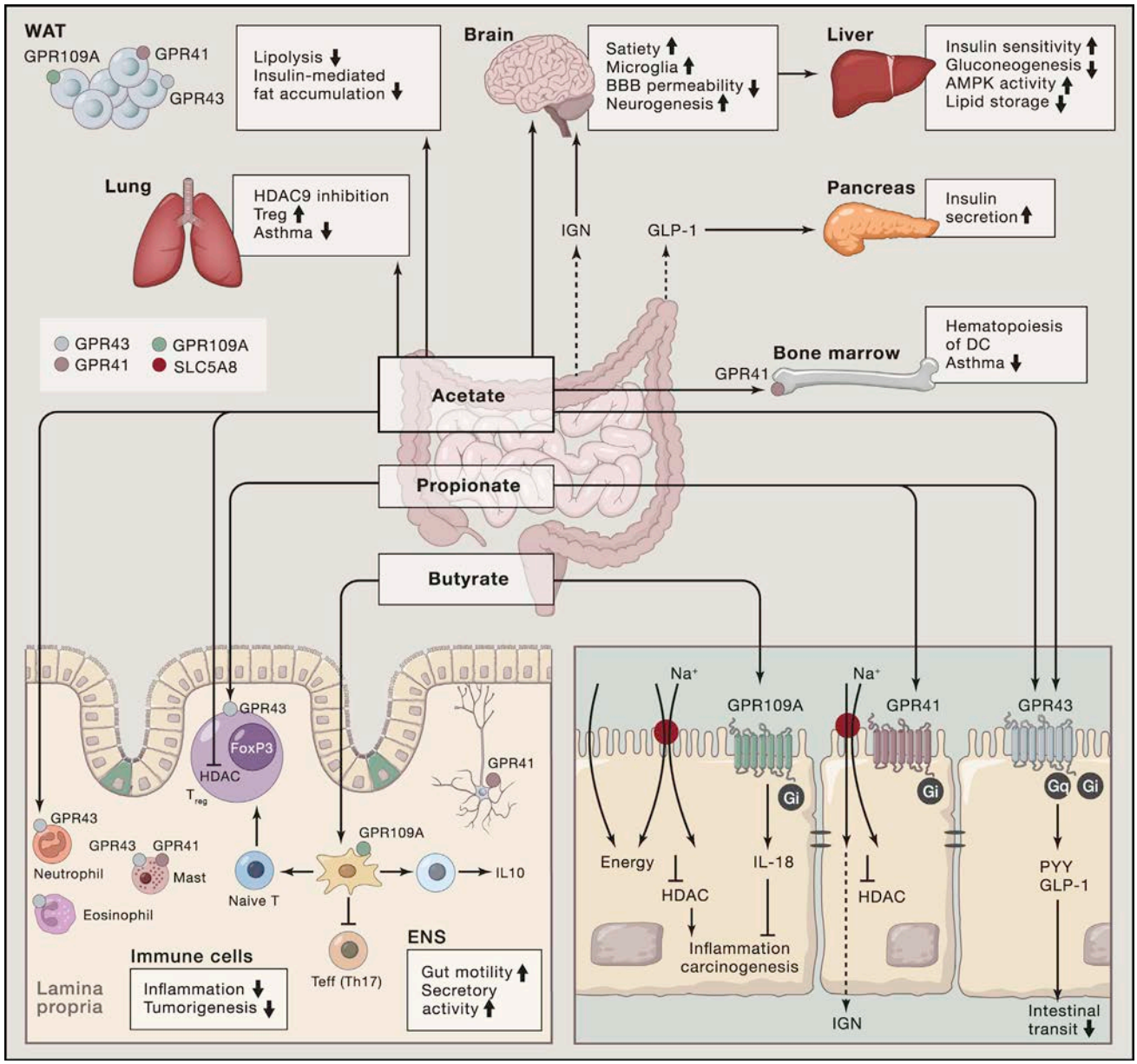

Figure 2. Mechanism of Action of Microbially Produced SCFAs

Fermentation of dietary fiber leads to the production of SCFAs via various biochemical pathways. The size of the letters symbolizes the ratio of SCFAs present. In the distal gut, SCFAs can enter the cells through diffusion or SLC5A8-mediated transport and act as an energy source or an HDAC inhibitor. Luminal acetate or propionate sensed by GPR41 and GPR43 releases PYY and GLP-1, affecting satiety and intestinal transit. Luminal butyrate exerts anti-inflammatory effects via GPR109A and HDAC inhibition. Furthermore, propionate can be converted into glucose by IGN, leading to satiety and decreased hepatic glucose production. SCFAs can also act on other sites in the gut, like the ENS, where they stimulate motility and secretory activity, or the immune cells in the lamina propria, where they reduce inflammation and tumorigenesis. Small amounts of SCFAs (mostly acetate and possibly propionate) reach the circulation and can also directly affect the adipose tissue, brain, and liver, inducing overall beneficial metabolic effects. Solid arrows indicate the direct action of each SCFA, and dashed arrows from the gut are indirect effects.

the epithelium. Furthermore, it is at present unclear whether GPR43 is expressed on the apical or basolateral side of the cell.

Outside of the gut, GPR43 seems to play an important role in white adipose tissue (WAT). *Gpr43*$^{-/-}$ mice are obese compared to their wild-type counterparts even on chow diet, whereas adipose-specific overexpression of *Gpr43* resulted in leaner mice. However, the effect was abrogated by antibiotic treatment, demonstrating the importance of microbial metabolism in forming ligands for adipose GPR43 signaling (Kimura et al., 2013). Indeed, acetate may be a functionally relevant metabolite, as it promotes anti-lipolytic activity

through GPR43 in WAT (Robertson et al., 2005). Acetate-dependent GPR43 stimulation in the WAT, but not in muscles or liver, also improved glucose and lipid metabolism (Kimura et al., 2013). Taken together, these data suggest that acetate may have metabolically beneficial effects through GPR43 activation in WAT. However, it should be noted that, in one study, *Gpr43* deficiency was associated with improved metabolic phenotypes (Bjursell et al., 2011). The reason for this discrepancy is currently unclear.

In contrast to GPR43, GPR41/FFAR3 couples only to G_i and is activated in the affinity order propionate>butyrate>>acetate with EC_{50} for propionate around 12–274 μM (Le Poul et al., 2003) (Table 2). However, interspecies variability exists—e.g., acetate was equipotent with mouse (m) GPR43 and mGPR41 (Hudson et al., 2012). Interestingly, GPR41 has been associated with microbial-induced adiposity, since conventionally raised *Gpr41*$^{-/-}$ mice are leaner than their wild-type counterparts, whereas this difference is abrogated under germ-free (GF) conditions. Furthermore, the microbiota, and presumably the resulting SCFAs, induced peptide YY (PYY) production in a GPR41-dependent fashion (Samuel et al., 2008). Thus, it is becoming increasingly clear that SCFA signaling through GPCRs in mice have profound effects on metabolism, but the role of GPR41/43 signaling in humans needs to be clarified.

A third GPCR, GPR109A/HCA2, responds to butyrate in an immune context and thus will be discussed below.

SCFAs in Health and Disease

Host Metabolism

Dietary fiber promotes weight loss and improves glycemic control, and several studies have sought to determine the impact of an SCFA-enriched diet to establish a direct causal relationship between fiber fermentation and improved metabolism. Mice fed a butyrate-enriched high-fat diet have increased thermogenesis and energy expenditure and are resistant to obesity (Gao et al., 2009). In the same manner, oral acetate gavage in an obese and diabetic strain of rats reduced weight gain and improved glucose tolerance (Yamashita et al., 2007). Other studies showed that supplementation with propionate or butyrate separately improved glucose homeostasis in rodents (De Vadder et al., 2014; Lin et al., 2012). In humans, acute administration of the inulin-propionate ester, which can be metabolized by the microbiota to propionate in the colon, significantly increased postprandial GLP-1 and PYY while reducing calorie intake at a buffet meal. Furthermore, after a long-term supplementation, this resulted in a significant reduction in weight gain (Chambers et al., 2015). Plasma concentration of PYY and GLP-1 is increased by rectal and intravenous perfusions of acetate in human subjects (Freeland and Wolever, 2010), and propionate supplementation in healthy women for 7 weeks reduced fasting glucose levels and increased insulin release during oral glucose tolerance test (Venter et al., 1990), suggesting a link between SCFAs, enteroendocrine hormones, and glucose homeostasis.

Recently, intestinal gluconeogenesis (IGN) was suggested to mediate beneficial metabolic effects by butyrate and propionate (De Vadder et al., 2014). Propionate is classically described as an efficient hepatic gluconeogenic substrate, but it also serves as a gluconeogenic substrate in the intestine before reaching the liver. Butyrate also induced IGN but did so by increasing concentration of cAMP in colonocytes. Thus, some of the beneficial metabolic effects induced by propionate and butyrate are mediated by de-novo-synthesized glucose from the gut epithelium, which is sensed in the portal vein and signals though a gut-brain neural circuit to increase insulin sensitivity and glucose tolerance (De Vadder et al., 2014).

Despite the fact that SCFAs classically have been associated with metabolic benefits and leanness (Ridaura et al., 2013), SCFA concentrations are increased in feces of obese humans compared to lean controls (Schwiertz et al., 2010). SCFA may constitute an important energy source in humans (Bergman, 1990), and it has been suggested that increased energy harvest, associated with increased polysaccharide degradation in the gut, could contribute to the obese phenotype in genetically obese mice (Turnbaugh et al., 2006). However, it is at present unclear whether SCFAs contribute to obesity or just reflect the altered gut microbiota.

Gut Immunity

Because of the high bacterial density in the gut, our intestine is a unique immunological site where host-microbiota interaction occurs. Perturbation of the equilibrium between the host immune system and microbiota modulates inflammation and can contribute to IBD. The role of the microbiota on immunity has been reviewed recently (Kamada et al., 2013); thus, we will focus on SCFAs and their receptors or HDACs in immunity.

The intestinal immune system must constantly maintain a delicate balance between tolerance to commensals and immunity to pathogenic bacteria, staying hyporesponsive to commensals under steady state. Thus, immune-suppressive mechanisms are indispensable for intestinal homeostasis. This can be achieved by increased IL-18 secretion by intestinal epithelial cells (IECs) and generation of Tregs and IL-10-producing T cells via butyrate-stimulated signaling of GPR109A (Singh et al., 2014). Also, a recent study suggests that high-fiber diet-induced activation of GPR43 and GPR109A activates the NLRP3 inflammasome, which is critical for intestinal homeostasis (Macia et al., 2015). Considering the high expression of SCFA receptors in immune cells (Table 2), we speculate that these are important regulators of T cell function. Recent studies have shown the effects of SCFAs on Treg cell expansion/generation via SCFAs-GPCR or their HDAC-inhibiting ability (Figure 2 and 3) (Arpaia et al., 2013; Furusawa et al., 2013; Singh et al., 2014; Smith et al., 2013). Although accumulating evidence supports the specific role of SCFAs on Treg cells, the role of SCFAs on T cell differentiation into both effector and regulatory T cells has been recently described, related to either immunity or immune tolerance depending on the immunological milieu (Park et al., 2015). In contrast to the earlier study showing the expression of GPR43 in colonic Tregs and myeloid cells (Smith et al., 2013), Park and co-authors reported that T cells do not significantly express GPR43 and thus GPR43 is not functional in regulating cytokine expression in T cells, which is rather dependent on HDAC activity

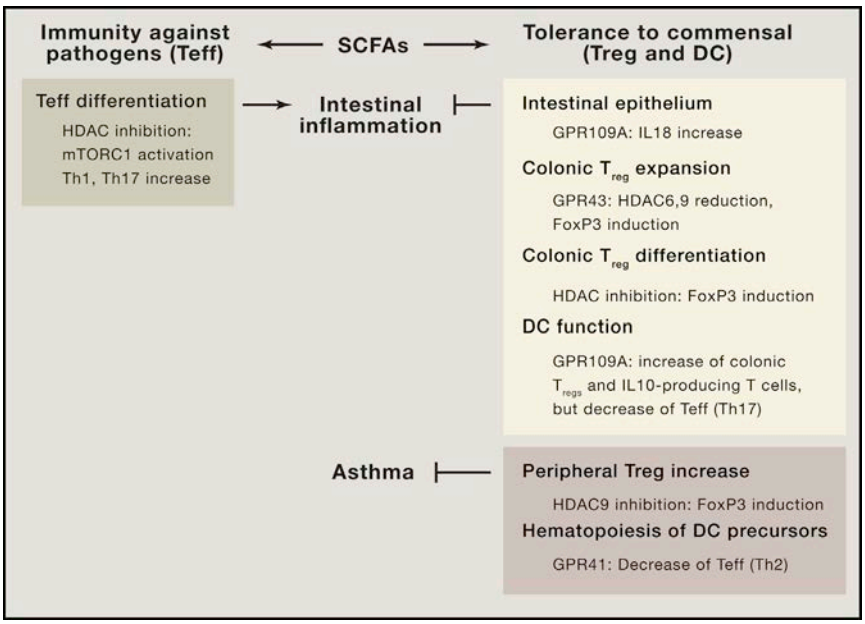

Figure 3. Impact of SCFA-Mediated Tolerance and Immunity in Intestinal and Allergic Airway Inflammation

To maintain homeostasis, our immune system should remain suppressive. Tolerance to commensals (tolerating self-molecules) is primarily achieved by IL-18 increase in intestinal epithelium and immune-suppressive Treg expansion/differentiation through Treg itself or DC. These effects are mediated by the interaction between SCFAs and their targets in the host (GPCR and/or HDAC), which is also important in the suppression of inflammation outside of the gut—dysregulation of immune tolerance can lead to allergic airway inflammation (asthma). However, host immune system should recognize and eliminate pathogens (non-self) by activating effector T cell functions, which are also known to be regulated by SCFA-mediated HDAC inhibition and mTORC1 activation, depending on immunological milieu.

(Park et al., 2015). Furthermore, they suggested that, if the host was in a situation of fighting against pathogens, SCFAs would facilitate differentiation of naive T cells into Th1 and Th17 cells to boost immunity. Collectively, SCFAs can modulate T cell function, but more research is required to pinpoint the underlying mechanism.

In terms of where signaling through GPR109A may occur, it is highly expressed on the lumen-facing apical membrane of colonic and small intestinal epithelial cells (Thangaraju et al., 2009). It is reasonable to consider other microbial metabolites as physiological ligands for GPR109A; theEC_{50} for butyrate on human and mouse orthologs is around 0.7 mM and 1.6 mM, respectively (Taggart et al., 2005). Since butyrate is produced in large quantities (mM) by bacterial fermentation of dietary fibers, it may be a physiologically relevant ligand for GPR109A in the gut. The relevance of GPR109A as a mediator of gut microbiota was supported by microbiota-dependent expression in the colon and ileum (Cresci et al., 2010), whereas it is unlikely to reach physiologically relevant levels in the periphery (~5 μM). Thus, many of the beneficial effects driven by butyrate-GPR109A likely occur in the colon.

Cancer

Less than 10% of all cancers are caused by germline mutations, and thus cancer is generally regarded as a disease of acquired somatic mutations and environmental factors. Recently, the gut microbiota has emerged as an environmental factor affecting host pathophysiology, with up to 20% of all cases of cancer worldwide associated with microbial infection (de Martel et al., 2012).

Chronic inflammation is a well-established risk factor for colorectal cancer (CRC) (Medzhitov, 2008). Pathogenic bacteria, but also commensal microbial elements, has been associated with inflammation and cancer development (Mazmanian et al., 2008). Commensal bacteria can promote as well as suppress colonic inflammation and cancer in a context-dependent fashion (Figure 4). Antibiotic treatment prevents chronic colitis, suggesting that normal colonic microbiota has a proinflammatory role (Videla et al., 1994). In contrast, GF and antibiotic-treated mice are more susceptible to dextran sulfate sodium (DSS)-induced colitis, which may be due to altered mucus quality. Activation of GPR43 by acetate markedly protected against gut inflammation in mice (Maslowski et al., 2009), proposing that normal microbiota-produced metabolites like SCFAs have a protective role in colonic inflammation. The expression of the SCFA receptors GPR109A and GPR43 is markedly reduced in colon cancer (Cresci et al., 2010; Tang et al., 2011), again supporting the protective role of SCFA signaling. More specifically, butyrate seems to be related to a protective role based on a significant decrease in the number of butyrate-producing bacteria in the colon of patients with ulcerative colitis and colon cancer (Frank et al., 2007; Wang et al., 2012) and an amelioration of experimental colitis (AOM (azoxymethane)/DSS treatment) through GPR109A (Singh et al., 2014). However, currently much remains unclear regarding the causal links between tumor-associated microbiota and metabolites in inflammation and cancer.

Butyrate can also promote tumorigenesis in a genetic mouse model with mutations in both the *Apc* gene and the mismatch repair gene *Msh2* ($Apc^{Min/+};Msh2^{-/-}$) (Belcheva et al., 2014). In this model, butyrate induced tumorigenesis independently of microbial-driven inflammation by instead inducing stem-cell-like characteristics in the crypts, possibly increasing the efficacy of stem cell generation and self-renewal (Liang et al., 2010). In this study, a low-carbohydrate diet was used, which not only reduces butyrate, but also glucose. Since cancer and stem cells exhibit great glucose dependency, some of the effects in the $Apc^{Min/+};Msh2^{-/-}$ mice may be attributable to reduced glucose availability. Taken together, when considering the effect of SCFAs on cancer, we need to consider genetic background, cellular energetics, and environmental contexts (i.e., inflammation or stem-cell-like character of the cell and the diet of the host).

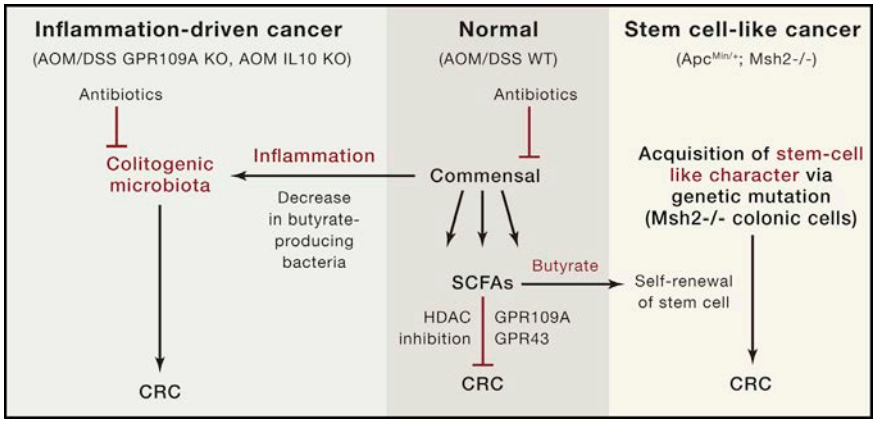

Figure 4. Context-Dependent Effects of Microbiota on CRC

Depending on the cellular context of the host (i.e., inflammation-driven or acquisition of stem-cell-like character), antibiotics and/or SCFAs can function as anti-inflammatory or pro-inflammatory. In normal conditions in which commensal-host interaction is nicely balanced, removal of commensals by antibiotics will erase the beneficial effect of SCFAs, contributing to CRC development.

Asthma

Like the intestinal epithelium, the airway epithelium forms a large interface between the external environment and the interior of the human body, with constant exposure to potential pathogens. Asthma is a chronic respiratory disease affecting 300 million people worldwide (Brusselle et al., 2013), characterized by airway hyper-reactivity and remodeling. Inadequate immune regulation and/or compromised airway epithelium result in an allergic airway disease, asthma (Holgate, 2011). A protective role of commensals and potentially their metabolites from asthma has been suggested (Russell et al., 2012).

A high-fiber diet (producing high amounts of acetate) suppresses allergic airway disease by enhancing regulatory T cells (Treg) through HDAC9 inhibition (Thorburn et al., 2015). High-fiber diet and subsequent propionate production can also protect against allergic airway by inducing hematopoiesis of dendritic cells that seed the lungs and reduce Th2 effector function in a GPR41-dependent fashion (Trompette et al., 2014). Similarly, intestinal helminth infection causes changes in commensal communities, resulting in an increase of SCFAs and reduction of allergic asthma in a GPR41-dependent manner (Zaiss et al., 2015). Thus, modulation of HDAC and GPR41-induced signaling can be important for shaping the immune niche in the lung and potentially other organs. An interesting future direction will be to understand whether the effects of SCFAs on circulating immune cells can be translated to human disease.

Nervous System

Besides its effects on intestinal epithelial cells, butyrate can also modulate the activity of the enteric nervous system (ENS) (Soret et al., 2010). For example, the SCFA receptor GPR41 is expressed in the ENS (Nøhr et al., 2013). A resistant starch diet (in which starch reaches the colon and can be considered a dietary fiber), intracecal butyrate infusion, and butyrate application to cultured myenteric ganglia all affect the ENS by increasing the proportion of cholinergic neurons translating to increased gut motility (Soret et al., 2010). In contrast to butyrate, propionate seems to decrease colon motility (Hurst et al., 2014). However, propionate increases secretory activity of the colon (Yajima et al., 2011) as well as the number of vasoactive intestinal peptide (VIP) neurons in the intestine (De Vadder et al., 2015).

Apart from the ENS, SCFAs also act on other peripheral neurons. In addition to the SCFA-GPR41 gut-brain neural axis responsible for improved energy metabolism discussed above (De Vadder et al., 2014), GPR41 is widely expressed in the peripheral nervous system, such as sympathetic ganglia, as well as vagal, dorsal root, and trigeminal ganglia (Kimura et al., 2011; Nøhr et al., 2015). Activation of GPR41 by SCFAs induces sympathetic activation via noradrenaline release, leading to increased energy expenditure and heart rate (Kimura et al., 2011), collectively suggesting profound effects of SCFAs in nervous signaling.

SCFAs can have various effects on the host brain. For example, when administered intravenously, a small fraction of acetate crosses the blood-brain barrier, where it is taken up and activates hypothalamic neurons driving satiety (Frost et al., 2014). A recent study explored a potential link between SCFAs and microglia maturation in the brain. Microglia are the resident macrophages of the brain and spinal cord, acting as the main form of immune defense in the central nervous system. Germ-free (GF) mice have defective microglia density in the brain. However, when GF mice were administered SCFAs in the water for 4 weeks, the number of microglia was restored, as was their function and morphology (Erny et al., 2015). This effect was dependent upon the activation of GPR43. Furthermore, SCFAs regulate the permeability of the blood-brain barrier (BBB). Colonization of GF mice with the butyrate producer *Clostridium tyrobutyricum* or with the acetate and propionate producer *Bacteroides thetaiotaomicron*, as well as oral gavage with sodium butyrate, decreases BBB permeability, associated with increased expression of occludin in the frontal cortex and hypothalamus (Braniste et al., 2014). Intravenous or intraperitoneal administration of sodium butyrate has been reported to prevent BBB breakdown and promote angiogenesis and neurogenesis (Kim et al., 2009; Yoo et al., 2011).

In summary, these data show that action of SCFAs is not limited to the gut. They can act at distal places such as the brain, modulating permeability, neurogenesis, and behavior of the host. Furthermore, they can also modulate autonomic functions independently of the central nervous system.

SCFA Precursors: Lactate and Succinate

Succinate and lactate are organic acids, which also are microbially produced in the gut but are usually considered as intermediates and are measured in lesser amounts mostly because of

consumption by other microbes that convert them to SCFAs (Cummings et al., 1987; Flint et al., 2012). However, microbially produced lactate and succinate may also have important signaling functions.

Lactate as Signaling Molecule

For about 4,000 years, people have been ingesting lactic acid bacteria with fermented and therefore preserved foods. Lactic acid bacteria are widespread in nature and also inhabit in the gastrointestinal tract (Garrote et al., 2015). Fermenting milk with lactic acid bacteria provides a final product that contains lactic acid, among other metabolites. Several studies demonstrate that lactic acid (lactate) can have diverse metabolic and regulatory properties, such as immune function, being an energy source for cell turnover, HDAC inhibitors, and signaling molecules.

In 2008 and 2009, two groups reported that L-lactate (2-hydroxypropanoate) is a natural ligand for G_i-coupled GPR81, inhibiting cAMP-mediated intracellular signaling events such as lipolysis. GPR81 is enriched in adipose tissue and was originally proposed as a potential target for treatment of dyslipidemia (Cai et al., 2008; Thangaraju et al., 2009). The EC_{50} value for L-lactate to GPR81 is around 5 mM but is more than 20 mM for D-lactate (Thangaraju et al., 2009). Whereas sufficiently high concentrations may be achieved upon exercise, microbially produced lactate is generally converted into propionate or butyrate by a subset of lactate-utilizing bacteria (Flint et al., 2012), and it is thus unlikely that bacterially derived lactate functions as a ligand for GPR81 outside of or even within the gut. In contrast, the vaginal microbiota produces a large quantity of lactate, i.e., vaginal secretions contain 10–50 mM lactate, of which ~55% is the D isoform (Boskey et al., 2001). Thus, microbially produced lactate may affect physiological functions in the vagina either through HDAC modulation or GPR81 signaling. Orally consumed probiotics like *Lactobacillus spp.* are believed to ascend to the vaginal tract (Reid et al., 2003), suggesting a gut-microbiota-mediated regulation of the vaginal microbiota.

Succinate as Signaling Molecule

Succinate is an important intermediate metabolite in the citric acid cycle, where it is formed from succinyl-CoA by succinyl-CoA synthetase and is converted to fumarate by succinate dehydrogenase, an oxygen-dependent enzyme. Gut microbiota can also produce considerable levels of succinate, but it is not clear whether microbially derived succinate acts as a signaling molecule. In humans, succinate concentration is 1–3 mM in the contents of large intestine and feces, which corresponds to about 2%–4% of the total concentration of organic anions (Meijer-Severs and van Santen, 1987). Succinate, mainly produced by *Prevotella*, activates dendritic cells (Rubic et al., 2008), and it will thus be interesting to determine whether microbially produced succinate modulates intestinal inflammation. This was supported by a study showing that polyphenols in conjunction with high-fat diet raise cecal succinate levels and inhibit growth and proliferation of colon cancer cells and angiogenesis (Haraguchi et al., 2014).

GPR91 was identified as a succinate receptor in 2004 (He et al., 2004), suggesting that microbially produced succinate may function as a signaling molecule. EC_{50} value of succinate on human and mouse GPR91 is 56 and 28 μM, respectively (He et al., 2004). Plasma succinate concentrations in rodents vary from 6 to 17 μM and 2 to 3 μM in humans (Ariza et al., 2012), suggesting that the levels in the gut, but not in the periphery, may be sufficient to activate GPR91. In summary, microbially produced succinate is associated with beneficial effects, but the exact role of succinate in modulating physiology, and whether it is dependent on GPR91, is currently unknown.

Conclusions and Outlook

Microbial interactions with dietary polysaccharides and the resulting SCFAs are important energy and signaling molecules. It is becoming increasingly accepted that butyrate-producing bacteria and butyrate per se may be beneficial for human health. However, it is unclear whether beneficial effects are driven by butyrate per se and/or in combination with other metabolites produced from these bacteria. It should be noted that the gut microbiota produces many other classes of metabolites such as bile acids and amino acid derivatives that may also have essential signaling functions.

Fermentative bacteria mostly target the colon, whereas effects of exogenously administered SCFAs may be dependent on route of administration and thus different from microbially produced metabolites. For example, oral delivery of butyrate may target the small intestine and reach supraphysiological concentrations in the periphery since it is not consumed by colonocytes. Tissue-specific effects of SCFAs have been demonstrated in the case of propionate, where propionate-dependent gluconeogenesis in the small intestine improves metabolic health, whereas hepatic gluconeogenesis is detrimental. Considering the expression of SCFA receptors in the small intestine, it will be important to understand SCFA production and their signaling in the small intestine using tissue- and even cell-specific knockout mice. Of course, studies with other microbial metabolites, such as lactate signaling in the vagina and succinate signaling in the gut, may also provide new and exciting possibilities for modulation of human health.

It will be a major challenge to identify the exact role of SCFAs in host (patho)physiology and to pinpoint their precise mechanisms, which can differ between tissues and even within the same tissue, depending on the cell type. Also, there is a relatively low specificity and affinity of microbial metabolites toward host targets (i.e., butyrate [mM range] versus niacin [nM range] for GPR109A). Thus, receptors recognizing microbial metabolites may originally have evolved to recognize endogenous molecules. However, there seems to be a selective pressure to sense microbial metabolites in the intestine during co-evolution between microbiota and host, as expression of SLC5A8 and GPR109A is increased in colonized mice. But because of the promiscuous nature between microbial metabolites and host targets, these metabolites might be able to exert broader impact on host pathophysiology.

Here, we discussed how SCFAs are synthesized, are distributed, and can signal and contribute to host physiology within the gut and in the periphery. However, their effects may well be exerted in a number of organs. Understanding spatiotemporal

concentration of metabolites and their functional capacity will hopefully lead to general principles for microbial metabolite actions affecting host health.

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