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Sphingolipids in host–microbial interactions

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Sphingolipids, a lipid class characterized by a long-chain amino alcohol backbone, serve vital structural and signaling roles in eukaryotes. Though eukaryotes produce sphingolipids, this capacity is phylogenetically highly restricted in Bacteria. Intriguingly, bacterial species commonly associated in high abundance with eukaryotic hosts include sphingolipid producers, such as the Bacteroidetes in the mammalian gut. To date, a role for bacterial sphingolipids in immune system maturation has been described, but their fate and impact in host physiology and metabolism remain to be elucidated. The structural conservation of bacterial sphingolipids with those produced by their mammalian hosts offer clues about which aspects of mammalian biology may be modulated by these intriguing lipids.

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Introduction

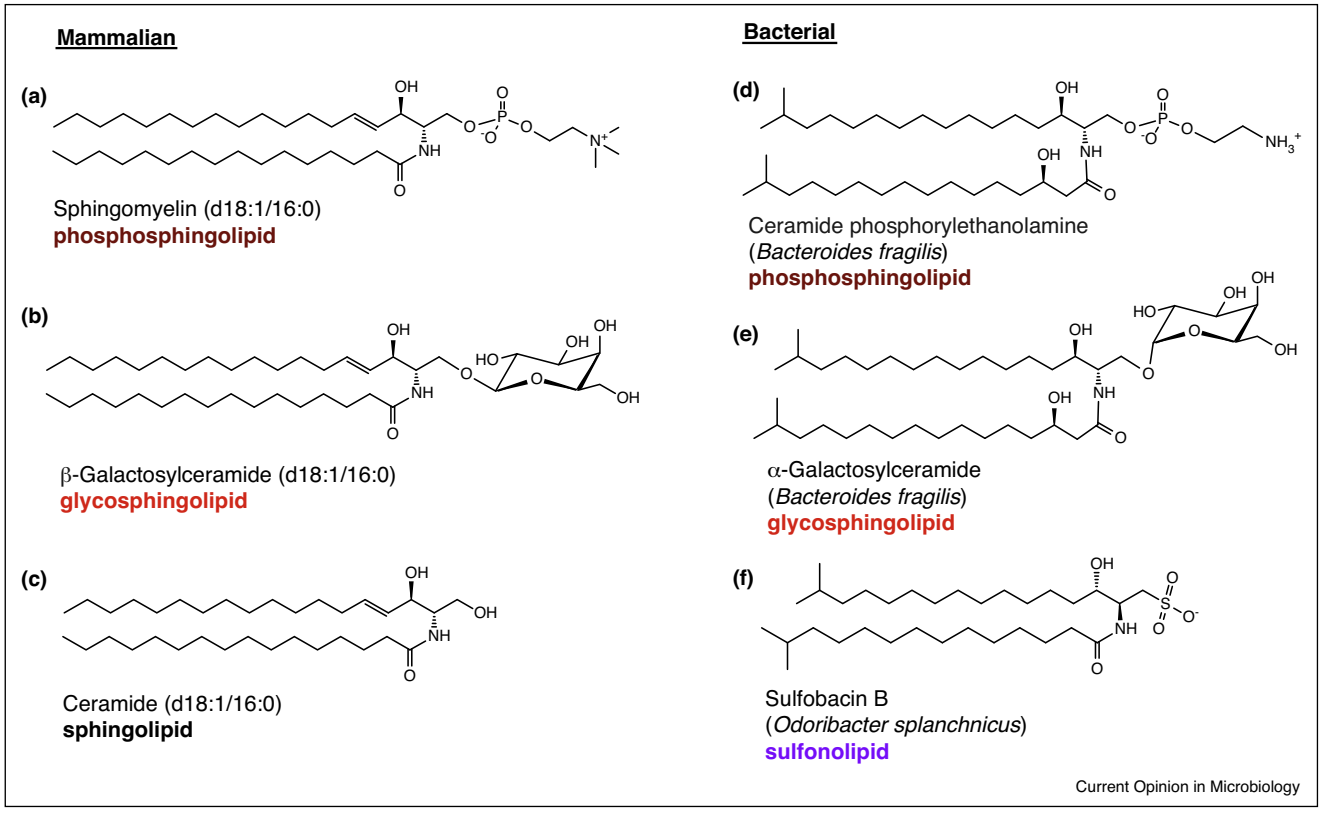
Sphingolipids are a class of lipids characterized by a long-chain amino alcohol sphingoid backbone with an amide-bound fatty acyl chain. Structural diversity arises through variation in the lipid headgroup (simple or branched sugar residues, or neutral or charged moieties) and sphingoid base/fatty acyl chain (length, degree of saturation, methylation) (Figure 1). Together, this variation produces thousands of unique sphingolipid structures [1]. The signaling and structural role conferred by each sphingolipid is highly specific, mediating numerous cellular processes in eukaryotes involved in apoptosis, cell differentiation, and inflammation [2].

Sphingolipid production is ubiquitous in eukaryotes, but the majority of Bacteria and Archaea are not known to produce sphingolipids. Amongst the Bacteria, known sphingolipid production is restricted to a small subset of the over 100 bacterial phyla in this domain. To date, known sphingolipid-producing bacteria include the majority of the Bacteroidetes phylum (e.g. genera such as *Bacteroides*, *Parabacteroides*, *Prevotella*, *Porphyromonas*, *Flectobacillus*) together with a few members of the Chlorobi phylum (e.g. *Chlorobium*). The Bacteroidetes and Chlorobi are sometimes referred to as a superphylum because they share a common ancestor [3], thus, sphingolipid production may be basal to this lineage. In addition to the Bacteroidetes/Chlorobi, a subset of Alpha-Proteobacteria (*Acetobacter*, *Sphingomonas*, *Novosphingobium*) and Delta-Proteobacteria (*Myxococcus*, *Bdellovibrio*) also produce sphingolipids. Despite the rarity of sphingolipid production in Bacteria, bacterial sphingolipids are found throughout nature, attesting to the success of sphingolipid-producers in the biosphere [4–6].

One intriguing aspect of the list of bacterial taxa known to produce sphingolipids is that many are known to associate with eukaryotic hosts. The Bacteroidetes phylum is dominant in the mammalian gut [7]. For instance, the human gastrointestinal tract is generally heavily colonized with members of the Bacteroidetes such as *Bacteroides* and *Prevotella* spp. (Figure 2) [8,9]. Additionally, some opportunistic pathogens in humans produce sphingolipids (e.g. *Sphingomonas* spp.) and are thought to derive from the plant rhizosphere [10]. Indeed, sphingolipid-producing members of the Proteobacteria have recently emerged as important colonizers of plants and animals. These include *Sphingomonas* spp. on plant and root surfaces [11,12] and *Acetobacter* spp. with *Drosophila melanogaster* and *Caenorhabditis elegans* [13–15]. The degree to which sphingolipid-production is a necessary element in the interactions of these bacteria and their hosts remains an open question.

Evidence for sphingolipid-mediated bacteria-host interactions starts at the root of the eukaryotic tree. The induction of multicellularity in the choanoflagellate *Salpingoeca rosetta*, a unicellular eukaryote, is driven by sulfonolipids (sulfur-inclusive sphingolipid-like lipids) produced by *Algoriphagus machus*, a Bacteroidetes sp. [16]. This process is suggested to be mediated by fusion of released bacterial outer membrane vesicles (OMVs) with the membrane of its recipient cell [17•]. As choanoflagellates are the closest living relatives of animal ancestors, this interaction suggests that sphingolipid-mediated interactions between bacteria and animals are basal traits. Indeed, the observation that sphingolipid-producing bacteria are abundant in the phyllosphere of plants [18] hints that sphingolipid production by bacteria may be important for a wide

Figure 1



Structural comparisons of select mammalian and bacterial sphingolipids. Bacteria can produce sphingolipids with odd-chain length, hydroxylated, or methylated sphingoid backbones and attached fatty acyl chains. However, bacterial sphingolipid headgroup variation encompasses the same lipid classes as in mammals, including phosphosphingolipids and glycosphingolipids. Structures from **(a–c)** LipidMaps [1], **(d,e)** Wieland [27], **(f)** Walker [28•].

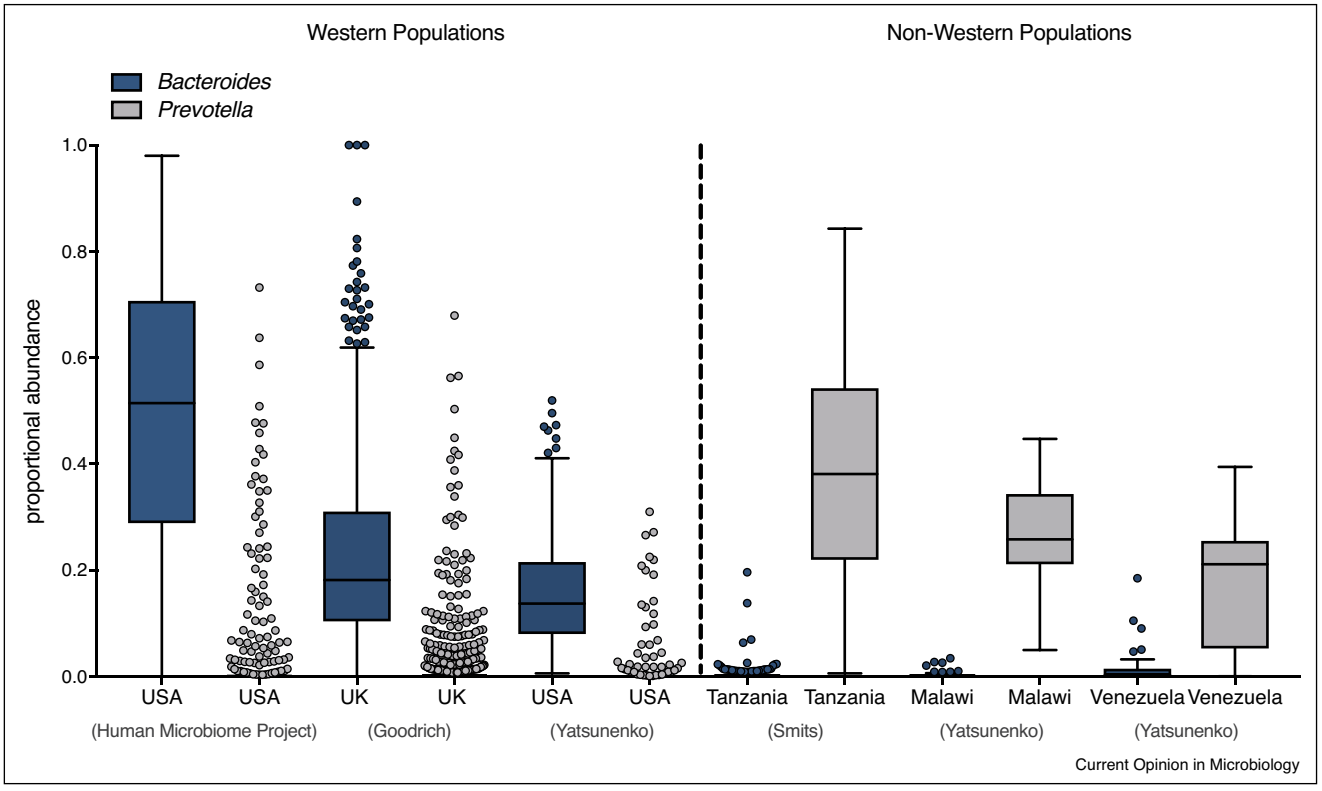
diversity of eukaryotes. Here, we review the biology of bacterial sphingolipid-production and producers in relation to mechanisms of host–microbial interactions, with a focus on human and mammalian biology.

Sphingolipid production by eukaryotes and bacteria

Bacteria and eukaryotes begin the process of sphingolipid production using the same enzymatic steps, but thereafter the pathways and products diverge. The initial step of sphingolipid synthesis involves the condensation of an amino acid (typically serine in mammals) and a fatty acid (typically palmitate in mammals) via the serine palmitoyltransferase (SPT) enzyme. In both eukaryotes and bacteria, *SPT* is highly conserved [19]. Indeed, bacterial proteins with high similarity to human SPT can be identified by homology from genomes of known sphingolipid-producing bacterial species. These include 2-amino-3-ketobutyrate CoA ligase and 8-amino-7-oxononanoate synthase, two genes likewise encoding acyltransferases (these also use glycine and alanine instead of serine; note that mammalian SPTs may also use these, but the resulting lipids are tied to neurotoxic effects [20,21]). Remarkably, the evolutionary history of the SPT homologs, as inferred from molecular phylogeny, mirrors the vertical ancestry (e.g. 16S rRNA gene phylogeny) of their hosts, with one exception (Figure 3). The Delta-Proteobacterium *Cystobacter fuscus* encodes an SPT more similar to those of the Bacteroidetes than to the other Delta-Proteobacteria, which we suggest indicates a lateral transfer event. But for the majority of the cases, the congruence of the 16S rRNA and SPT gene phylogenies implies vertical inheritance and high degree of conservation over time.

Eukarya and the bacteria also differ in their sphingolipodomes. Just as the majority of genes responsible for bacterial sphingolipid metabolism are unknown, the full diversity of bacterial sphingolipid structures is also less well-characterized than in their mammalian counterparts. Whereas mammals predominantly synthesize even-chained, linear sphingoid backbones, the sphingoid backbones and attached fatty acyl chains of bacterial sphingolipids are often odd-chain length, methylated, or hydroxylated (Figure 1) [22]. Additional structural variation arises from the head groups, which can include

Figure 2



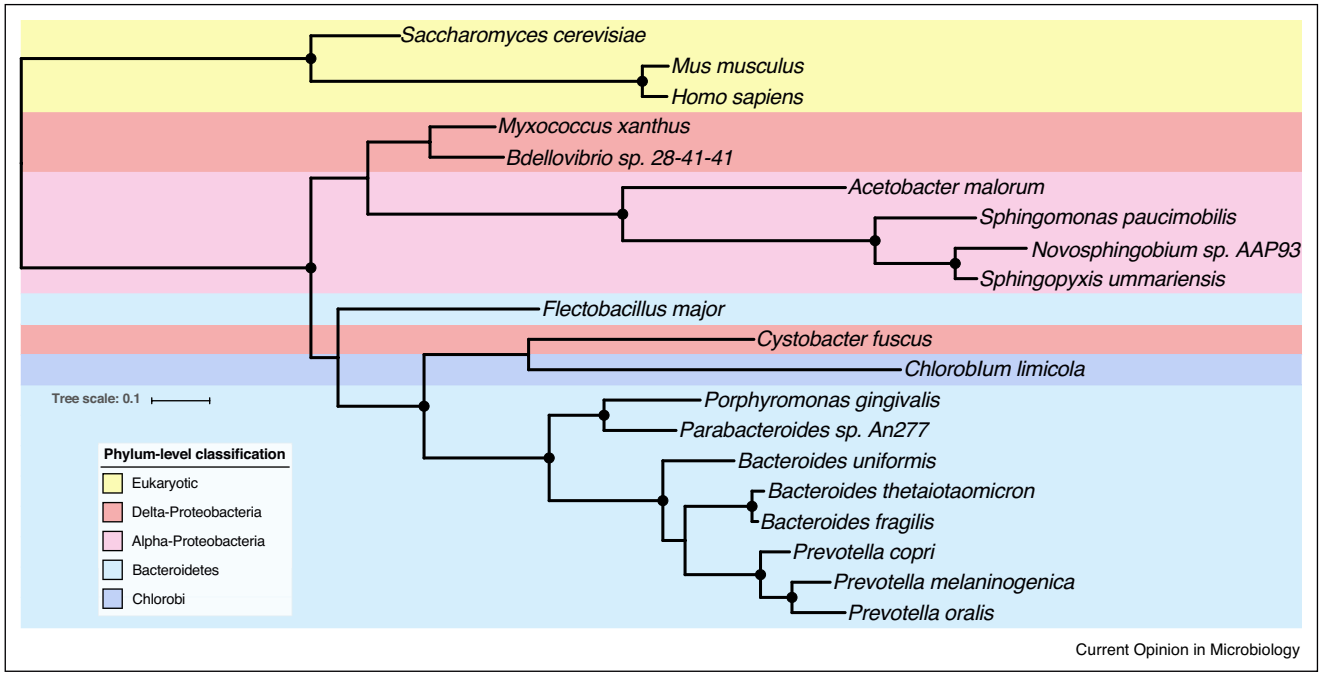
Bacteroides predominate in the Western gut microbiome, and *Prevotella* in the non-Western gut microbiome. Tukey boxplots display the distributions of proportional abundances of 16S rRNA gene sequences mapping to the *Bacteroides* (teal) or *Prevotella* (grey) genera and derived from fecal DNA obtained from Western and non-Western individuals in previously published studies. Datasets were obtained from the following studies: The Human Microbiome Project (HMP) [52], which included 385 samples from North American (USA) subjects; Goodrich *et al.* [53], with 1024 samples from the United Kingdom; Yatsunenko *et al.* [54], whose study included 228 samples from the USA, 54 samples from Malawi and 38 from Amerindians in Venezuela; and Smit *et al.*, including 350 samples from Hadza in Tanzania [9].

phosphorylethanolamine groups similar to human sphingomyelins, phosphorylglycerol or phosphorylinositol moieties, and simple or extensively chained glycan groups [23–25]. Within the human gut, members of the Bacteroidetes (e.g. *Bacteroides*, *Provotella*, *Porphyromonas*) are known to make sphingophospholipids resembling the sphingomyelin abundant in mammalian membranes, glycosphingolipids, and dihydroceramides [22,24,26,27]. Also within the Bacteroidetes phylum, *Alistipes* and *Odoribacter* spp. synthesize sulfonolipids [28•]. The structural conservation of sphingolipids between bacteria and their eukaryotic hosts raises the question of whether this association is driven in part by the exploitation of sphingolipid signaling pathways in their hosts.

Why do bacteria produce sphingolipids? One way sphingolipids may promote fitness (survival and replication) is through resistance to stress, particularly as related to membrane integrity. Studies with human gut Bacteroidetes have shown that SPT is necessary for stress resistance and prolonged survival. For instance, chemical inhibition and genetic knockouts of SPT orthologues in *Bacteroides fragilis* and *Porphyromonas gingivalis* produce cells with reduced resistance to oxidative stress and incapable of surviving stationary phase growth [26,29•]. Interestingly, membrane reconstitution of *B. fragilis* lipids indicated that cholesterol-enriched lipid microdomains, similar to those observed in eukaryotic cells, also form in the bacterial membrane in a sphingolipid-dependent manner. *B. fragilis* also has homologues of SPFH (stomatin, prohibitin, flotillin, HflK/C) domain proteins that are involved in eukaryotic sphingolipid microdomain formation. Together, these findings suggest that, like eukaryotes, *B. fragilis* may use a microdomain sphingolipid signaling pathway to respond to environmental stress [29•].

In non-host-associated taxa, sphingolipids may confer resistance to heat-stress in environmental habitats. For instance, Sollich and colleagues reported an enrichment of Bacteroidetes-like sphingolipids in hyperthermic marine sediments, and hypothesized that membrane

Figure 3



The molecular phylogeny the serine palmitoyl-transferase (SPT) gene and homologs encoded by eukaryotes and bacteria largely tracks their vertical lineage ancestry. Branching patterns of the SPT-homolog gene tree recapitulate the vertical ancestry of the host taxa, as indicated by the color background referring to the phylum-level classifications. Note that *Flectobacillus major* is basal within the Bacterdoidetes group, which is congruent with its classification in the Cytophagia class. The exception is *Cystobacter fuscus*, a member of the Delta-Proteobacteria, which is nested within the Bacteroidetes, suggesting a lateral gene transfer event. Labels refer to the taxa whose genomes encode the SPT homolog genes and the higher-level phylogeny is indicated by the color of the background: Eukaryotes are represented by *Saccharomyces cerevisiae*, *Mus musculus* and *Homo sapiens*; the Delta Proteobacteria by *Myxococcus xanthus*, *Bdellovibrio sp. 28-41-41* and *Cystobacter fuscus*; Alpha-Proteobacteria by *Acetobacter malorum*, *Sphingomonas paucimobilis*, *Novosphingobium sp.AAP93* and *Sphingopyxis ummariensis*; the Chlorobi by *Chlorobium limicola*; the Cytophagia class of the Bacteroidetes by *Flectobacillus major*, and the Bacteroidia class of the Bacteroidetes by *Porphyromonas gingivalis*, *Parabacteroides sp. An277*, *Bacteroides uniformis*, *Bacteroides thetaiotaomicron*, *Bacteroides fragilis*, *Prevotella copri*, *Prevotella melaninogenica*, and *Prevotella oralis*. Amino acid sequences were aligned using Clustal Omega and the maximum likelihood tree was generated in RaxML (ProtCat substitution model, Dayhoff matrix, hill-climbing algorithm; eukaryotes as outgroup). Annotations identified using NCBI-BLASTP include SPT (all eukaryotes and *B. fragilis*), 2-amino-3-ketobutyrate CoA ligase (*P. copri*, *P. oralis* and *C. limicola*), a hypothetical protein (*Bdellovibrio* sp. 28-41-41) and 8-amino-7-oxononanoate synthase (all others). Circles indicate nodes with minimum 85% bootstrap support. Scale bar is amino acid substitutions per site.

sphingolipids facilitate tight lipid packaging and confer increased stability and rigidity to the membrane, in part due to the increased hydrogen bonding potential of the amino groups [6]. Although sphingolipid production in bacterial inhabitants of eukaryotic surfaces may have its origin in resistance to stress, this capacity may be maintained by host selection. During periods of fasting, the sphingolipid-rich *Bacteroides* are known to preferentially remain in the gut by switching their glycan foraging to host mucus [30]. If sphingolipids from these bacteria enter the host (see below), then it is possible that the host derives sphingolipids from the gut even during periods of food deprivation.

Bacterial sphingolipids in mammalian immune regulation

To date, the best-characterized sphingolipid-mediated bacterial–mammalian interactions involve modulation of the host's immune system. For instance, *P. gingivalis*, a resident of the human mouth implicated in the etiology of periodontal disease, relies in part on sphingolipid production for its virulence [26]. In another example, the membranes of some *Sphingomonas* spp. lack the lipopolysaccharide characteristic of Gram-negative bacteria, instead containing glycosphingolipids [31]. These glycosphingolipids are sufficient for recognition by the host independently of Toll-like receptor signaling: they activate natural killer T (NKT) cells and lead to rapid cytokine release, facilitating bacterial clearance during infection [31,32]. Though in *Sphingomonas* spp. bacterial sphingolipids are essential for host recognition of surface polysaccharides, many other pathogens instead rely on host sphingolipids to promote their virulence. These include, for example, the binding of bacterial toxins to glycosphingolipids (e.g. botulism, cholera), endocytosis into macrophages through ceramide-rich rafts (e.g. *Salmonella typhimurium*, *Shigella*

flexneri, *Mycobacterium* spp.), and scavenging of host sphingolipids (e.g. *Chlamydia*) (reviewed in [33]).

These sphingolipid–immune interactions are not limited to pathogens. Recent work has highlighted such an interaction between the common gut commensal *B. fragilis* and the host. This species also synthesizes glycosphingolipids in the form of α-galactosylceramide. Though the α-galactosylceramide of *B. fragilis* is structurally similar to the potent NKT-activator KRN7000 (a synthetic specific ligand for human and mouse NKT cells) [27], the bacterial lipid has a shorter, methylated sphingoid backbone suggested to act as an antagonistic ligand or occupy the binding space of CD1 proteins, which present these lipid antigens to NKT cells [34•]. An and colleagues showed that pups of mice mono-colonized with wild-type *B. fragilis* had reduced colonic invariant NKT (iNKT) cells in adulthood and were protected against induced colitis; these effects were dependent on the presentation of *B. fragilis* glycosphingolipids in early development [34•]. Of the sphingolipids produced by *B. fragilis*, only α-galactosylceramide had these effects, which emphasizes how seemingly minor structural variations can confer specific, even opposite effects [34•].

Another way bacterial sphingolipids may interact with the host immune system is via induction of antibodies. An example of this comes from a study of *Flectobacillus major* (Bacteroidetes) with rainbow trout. *F. major* is a skin-associated and gill-associated symbiont. Sepahi *et al.* showed with *in vitro* experiments that its constituent sphingolipids induce B cell differentiation [35].

Bacterial sphingolipids in the gut

The Bacteroidetes phylum dominates the human gut, with *Bacteroides* or *Prevotella* comprising on average 30–50% of the fecal microbiome in Western and non-Western populations (Figure 2, for a similar comparison with more populations, see [9]). The total contribution of bacteria to host sphingolipid pools remains to be characterized, but the amount of sphingolipids present in microbial cells in the gut can be estimated. A human colonic microbiome consisting of 200 g of bacterial cells [36], a third of which are *Bacteroides*, will contain, based on cell hydrolyzable lipid content estimates [22], roughly 1 g of intestinal bacterial sphingolipids at a given time, in addition to the sphingolipids continuously released from these cells in OMVs [17•]. Thus, a majority of the human population carries within the gut an endogenous source of sphingolipids.

Work in mice has shown that sphingolipids derived from the gut can be traced to organs throughout the body. Fukami *et al.* showed that sphingolipids extracted from *Acetobacter malorum* and orally introduced into mice were readily uptaken and metabolized into complex sphingolipids in the liver [37]. Additionally, these lipids were located in tissue as far ranging as the brain and skin, providing evidence that intestinal bacterial sphingolipids are readily used by the host in sphingolipid homeostasis. These findings, together with the observations that specific sphingolipids made by bacteria in the gut interact with iNKT cells in the lamina propria (see above), demonstrate that bacterial sphingolipids move into the host.

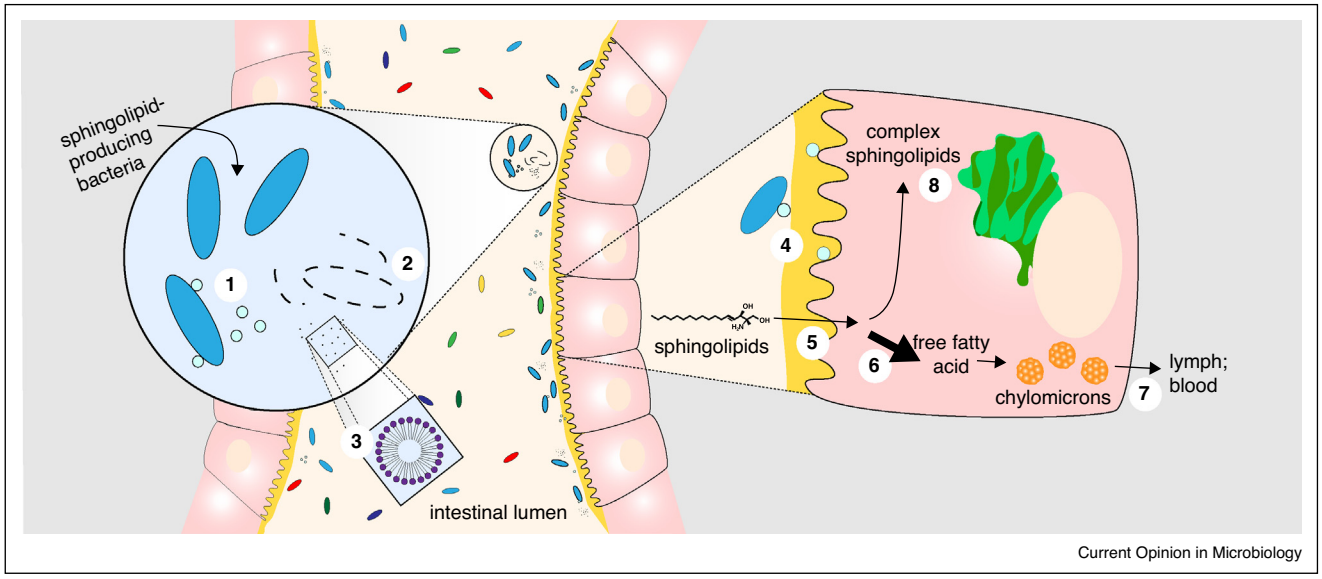
But how does this occur, and which lipids travel? Figure 4 shows a diagram of the possible ways in which sphingolipids may travel from bacterial cells into the host. One way that these lipids may be delivered is via OMVs, which are composed of the sphingolipid-rich outer membrane of the cell, and fuse with, or are endocytosed by, eukaryotic membranes [17•]. For instance, *Bacteroides* OMVs cross the intestinal mucus layer to reach the underlying cells and elicit immune-related host effects [38]. As an alternative to OMV delivery, normal bacterial cell turnover may deliver free lipids in small liposome or micelle-like structures to intestinal epithelial cells, and passive diffusion can then transport individual lipids across the cell membrane.

Fate of bacterial sphingolipid metabolites

Complex bacterial sphingolipids, like mammalian sphingolipids (including sphingomyelin, glycosylated sphingolipids, and ceramide), cannot passively diffuse into intestinal enterocytes due to their size and polarity. For them to passively diffuse, these complex lipids must first be hydrolyzed by bile-associated and enterocyte ectoenzymes into their sphingosine backbones, constituent fatty acids, and attached head groups [39]. Within intestinal enterocytes, the majority of sphingosine is degraded, incorporated into triglycerides, and enters the general circulation in chylomicrons. A small percentage of these absorbed sphingoid bases are re-synthesized into ceramide and more complex sphingolipids in the endoplasmic reticulum and Golgi apparatus [40].

Absorbed mammalian sphingolipid metabolites influence enterocyte fatty acid flux, and it is reasonable to assume that bacterial sphingolipid metabolites would also contribute to and stimulate lipid metabolic pathways. In a state of over-nutrition, lipids accumulate in non-adipose tissues (e.g. liver, skeletal muscle) and excess saturated fatty acids drive *de novo* sphingolipid synthesis, causing a ceramide-dependent inhibition of insulin sensitivity [41,42]. The majority of dietary sphingolipids, after metabolism and uptake, are converted to palmitate and other fatty acids in enterocytes; therefore, the absorption of bacterial sphingolipid metabolites may influence this flux. Palmitate levels are tightly regulated in the cell, with physiological levels of palmitoyl-CoA maintained around the Km for SPT, the rate-limiting enzyme for *de novo* sphingolipid synthesis [43]. As such, seemingly minor bacterial contributions to the palmitoyl-CoA pool may alter host rates of sphingolipid synthesis, potentially leading to altered ceramide metabolism in the liver and skeletal muscle [44].

Figure 4



Model of the mechanisms through which gut-associated sphingolipid-producing bacteria may influence host intestinal cells. In this cartoon, a single layer of epithelial cells is shown flanking either side of the gut lumen, which is populated by bacteria. On the left, the blue circle shows an enlarged area to highlight the following: (1) outer-membrane vesicles contain lipids from the outer bacterial membrane leaflet, including sphingolipids in sphingolipid-producing species; these are known to reach the intestinal epithelial cell layer; (2) bacterial cell turnover can provide membrane sphingolipids that are digested extracellularly; and (3) in the aqueous lumen, lipids form micelles and liposomes. On the right, an enlarged view of a single enterocyte displays: (4) sphingolipid delivery may take place by OMV uptake, or by (5) passive diffusion of simple sphingolipid metabolites across the cell membrane; (6) dietary sphingolipids, once absorbed by an enterocyte, are most often converted to free fatty acids and incorporated into triglycerides in chylomicrons, where the lipids enter the general circulation (7). Alternatively, a small portion of these sphingoid backbones are known to be repurposed into more complex sphingolipids by the host cell (8). Further research is required to confirm these mechanisms.

Alternatively, the repurposing of bacterial sphingoid backbones in host enterocytes may competitively inhibit the *de novo* synthesis of sphingolipids.

Potential fate of unhydrolyzed bacterial sphingolipids

Whereas dietary mammalian sphingolipids described above are broken down and taken up in the small intestine, the majority of sphingolipid-producing bacteria reside in the large colon. Here, sphingomyelin-like bacterial lipids would be subjected to lower levels of host sphingolipid degradation enzymes than in the small intestine. In addition, sphingomyelin hydrolysis is generally slow [40]. Though it is likely that the microbial community in the gut contributes substantially to the degradation of these lipids [45], a fraction will remain in their complex, unhydrolyzed forms. Thus, it is likely that in the colon, bacterial sphingolipids are present in both complex and hydrolyzed forms.

Based on the structural similarity of *Bacteroides* sphingolipids to the more complex mammalian sphingolipid sphingomyelin (Figure 1), it is tempting to suggest that these could likewise influence lipid absorption and lead to systemic changes in lipid metabolism. In animal studies, dietary (animal source) sphingomyelin supplementation has been shown to reduce lipid absorption and reduce cholesterol absorption by limiting its solubilization [46,47]. Furthermore, sphingomyelin supplementation in mice fed a high-fat diet has been shown to attenuate hepatic steatosis, reduce hepatic triglyceride levels, and reduce hepatic expression of PPARγ genes [48]. The observation that sphingomyelin supplementation to a high-fat diet can ameliorate its negative impact on metabolism in mice raises the question of whether bacterial sphingolipids can play a similar a role in ameliorating the negative metabolic effects of a Western diet in humans.

Potential for bacterial sphingolipid mediation of mammalian signaling pathways

Bacterial sphingolipids and their metabolites may be sensed at the level of intestinal epithelial cells, eliciting broader systemic host effects. Sphingolipid metabolites (e.g. ceramide, sphingosine-1-phosphate) are potent signaling molecules regulating diverse cell processes in the host including cell proliferation, apoptosis, differentiation, and inflammation (among other roles) [2]. Sphingosine-1-phosphate (S1P), for instance, is recognized by G-protein-coupled receptors (GPCRs), including those in the endothelial cells of the intestinal lamina propria [49,50]; in a gut

microbiome rich in sphingolipid-producers, intestinal sphingolipid-producing bacteria may contribute S1P-like metabolites that interact with these receptors. A precedent exists for the agonism of host intestinal GPCRs by lipids from gut bacteria. *N*-acyl amides, fatty acids each with an acyl group and nitrogen moiety (and thus structurally very similar to sphingolipids) are produced by mammalian gut bacteria and act as specific ligands for mammalian GPCRs, including the S1P receptor S1PR4 [51•]. These bacterial fatty acids are able to mediate host metabolism–blood glucose levels decreased in mice colonized with bacteria engineered to inducibly express an *N*-acyl amide acting as a ligand for another host-GCPR implicated in glucose homeostasis (GPR119) [51•]. The ability of bacterial lipids to act as signaling molecules mediating host metabolism and other processes, particularly in the context of sphingolipids, warrants further exploration.

Conclusions

Although common in eukaryotes, bacterial sphingolipid production is phylogenetically restricted mainly to members of the Bacteroidetes and selected Proteobacteria. These species are often found associated with a diverse range of eukaryotic hosts, in which they are known to influence host immune responses. In the mammalian gut, sphingolipid-producing *Bacteroides* and *Prevotella* spp. are abundant, providing an endogenous pool of bacterial lipids. The structural similarity of bacterial and eukaryotic sphingolipids suggests a possible mechanism for bacterial influence on their mammalian hosts, with potential mediation of the sphingolipid signaling pathways ubiquitous in mammals or alterations to host lipid metabolism. Further research should include the following areas: first, a better understanding of the diversity of sphingolipids produced by various gut commensal bacteria, second, how these initial structures produced by the bacteria are metabolized, taken up, and clarification of the role of OMVs in this process, third, a better understanding of the contribution of bacterial sphingolipids to host metabolism and their potential role as host signaling molecules, and fourth, the role of bacterial sphingolipids in progressive diseases in which sphingolipids are implicated, such as cancer and inflammatory bowel disease.

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- • of special interest
- •• of outstanding interest

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