Bacterial Sphingolipids and Sulfonolipids

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Abstract

The bacterial envelope is often composed by two membranes: the inner or cytoplasmic membrane and an outer membrane. The inner membrane consists of a lipid bilayer with phospholipids covering the inner and the outer leaflet. Although the outer membrane displays a bilayer structure as well, only the inner leaflet of the outer membrane is composed of phospholipids, whereas the outer leaflet is typically formed by lipid A-containing lipopolysaccharides in Gram-negative bacteria. However, some bacteria lack lipopolysaccharides and have sphingolipids in the outer leaflet of their outer membrane instead. Sphingolipids are considered to be typical eukaryotic membrane lipids, essential components of

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the plasma membrane, and are crucial for signaling and organization of lipid rafts in eukaryotes.

Although there is a considerable structural diversity within bacterial sphingolipids, very little is known about their biosynthesis, transport to the outer leaflet of the outer membrane, or their evolutionary history. Whereas bacterial sphingolipids seem to be important as an outermost protective layer in some bacteria, for the survival of symbiotic *Bacteroides* in humans, as virulence factors in some pathogenic bacteria, and maybe in fruiting body formation in myxobacteria, their biological functions are poorly understood on a molecular level.

Sulfonolipids are structural analogues of sphingolipids and seem to be important for gliding motility in *Cytophaga* species, but also as crucial bacterial factors that trigger multicellularity in choanoflagellates, the closest living relatives of animals.

1 Introduction

Sphingolipids (SphLs) are membrane-forming lipids and exist in most membranes of eukaryotes. In plasma membranes, they occur mainly in the outer leaflet and they play crucial roles in signaling and organizing lipid rafts (Nelson and Cox 2017). Members of the sphingolipid family of lipids, including sphingoid bases, sphingoid base phosphates, ceramides, and complex sphingolipids, serve vital functions in cell biology and are involved in the regulation of cell division, differentiation, migration, programmed cell death, and other processes (Nelson and Cox 2017). Sphingolipid biosynthesis, regulation, and function have been studied extensively in eukaryotic microorganisms, i.e., yeast, and these findings have been reviewed in some detail (Cowart and Obeid 2006; see Ren and Hannun, "▶ Chap. 18, "Metabolism and Roles of Sphingolipids in Yeast *Saccharomyces cerevisiae*," this volume).

Many Gram-negative bacteria possess two bilayered membranes in their envelope: the inner or cytoplasmic membrane (IM) and the outer membrane (OM). The IM is composed of (glycero)phospholipids (PLs) and proteins, whereas the OM harbors a different set of proteins, PLs at its inner leaflet, and the lipid A moiety of lipopolysaccharides (LPS) at its outer leaflet (Raetz and Dowhan 1990). However, since the early 1990s, it is clear that some Gram-negative bacteria lack LPS and seem to have SphLs instead in the outer leaflet of their OM (Kawazaki et al. 1994).

Other bacteria, i.e., the genus *Acetobacter*, may have both LPS and SphLs in their OM. In *Acetobacter malorum*, an increase of ceramide can be observed in acidic conditions of growth or at elevated temperatures (Ogawa et al. 2010), which suggests that under these conditions of stress, LPS is at least partially replaced by SphLs. Bacteria of the *Bacteroides* genus are predominantly in the human intestine, and many *Bacteroides* species have in addition to LPS (Weintraub et al. 1989) distinct phosphosphingolipids (Kato et al. 1995).

Overall, SphLs seem to occur only in few bacteria, particularly some anaerobes, where they functionally replace other bacterial membrane lipids. SphLs are found in the genera *Pedobacter* (Steyn et al. 1998), *Bacteroides*, *Prevotella*, *Porphyromonas*, *Fusobacterium*, *Sphingomonas*, *Sphingobacterium*, *Bdellovibrio*, *Cystobacter*,

Mycoplasma, *Flectobacillus*, *Acetobacter* (Olsen and Jantzen 2001), *Bacteriovorax* (Jayasimhulu et al. 2007), *Sorangium* (Keck et al. 2011), and *Myxococcus* (Lorenzen et al. 2014). Initially, their occurrence in bacteria was thought so unusual that often the genus name of the respective bacterium harbors the prefix "Sphingo," i.e., in *Sphingomonas* and *Sphingobacterium*. However, as outlined below, SphLs might be more widespread in bacteria than originally thought. In this chapter, we cover structures of SphLs encountered in bacteria, present knowledge on bacterial SphLs biosynthesis, localization, as well as transport, and we report on their functions. We also give a brief overview on sulfonolipids, structural analogs of SphLs.

2 Bacterial Sphingolipids

2.1 Structures of Bacterial Sphingolipids

Some Gram-negative bacteria, such as *Sphingomonas capsulata*, lack LPS in their outer membrane and instead have glycosphingolipids (glyco-SphLs) as functional replacements. In *Sphingomonas paucomobilis*, two glyco-SphLs differing in their ceramide structures are substituted with the tetrasaccharide Man-Gal-GlcN-GlcA (Kawahara et al. 1991) (Fig. 1). The chirality at carbon atoms C-2 and C-3 of the sphingoid base is D-erythro (Olsen and Jantzen 2001; Hanada 2003). The variability of glyco-SphLs in the outer bacterial membrane of the *Sphingomonadaceae* is considerable (Kinjo et al. 2008), and only some of these glyco-SphLs, such as GSL-1 (Fig. 1), are recognized by natural killer T cells which provide an innate-type immune response towards glyco-SphL-containing bacteria (Kinjo et al. 2008; Wu et al. 2006). Major molecular species of ceramides in sphingobacteria have been identified as 2-*N*-2′-hydroxy-13′-methyltetradecanoyl-15-methylhexadecasphinganine, 2-*N*-13′-methyltetradecanoyl-15-methylhexadecasphinganine, and 2-*N*-13′-methyltetradecanoyl-hexadecasphinganine (Yano et al. 1982, 1983; Naka et al. 2003). Many *Bacteroides* species have two types of phosphosphingolipids, ceramide phosphoethanolamine, and ceramide phosphoglycerol (Kato et al. 1995).

Apparently, sphingolipids seem to be common components of myxobacterial membranes as ceramides were found in *Myxococcus xanthus* and in *Cystobacter fuscus*, ceramide phosphoinositol in *M. xanthus*, ceramide phosphoethanolamine in *Myxococcus stipitatus* (Lorenzen et al. 2014), β-D-glucosylsphingenine, and even phosphonosphingolipids in *Sorangium cellulosum* (Keck et al. 2011). Also, *Bacteriovorax* (formerly *Bdellovibrio*) *stolpii*, which lives as a parasite in the periplasmic space of larger Gram-negative bacteria, possesses an ample spectrum of phosphonosphingolipids (Jayasimhulu et al. 2007).

2.2 Biosynthesis of Bacterial Sphingolipids

In eukaryotes, the biosynthesis of sphingolipids takes place in five stages (Nelson and Cox 2017). It begins with the condensation of serine and a fatty

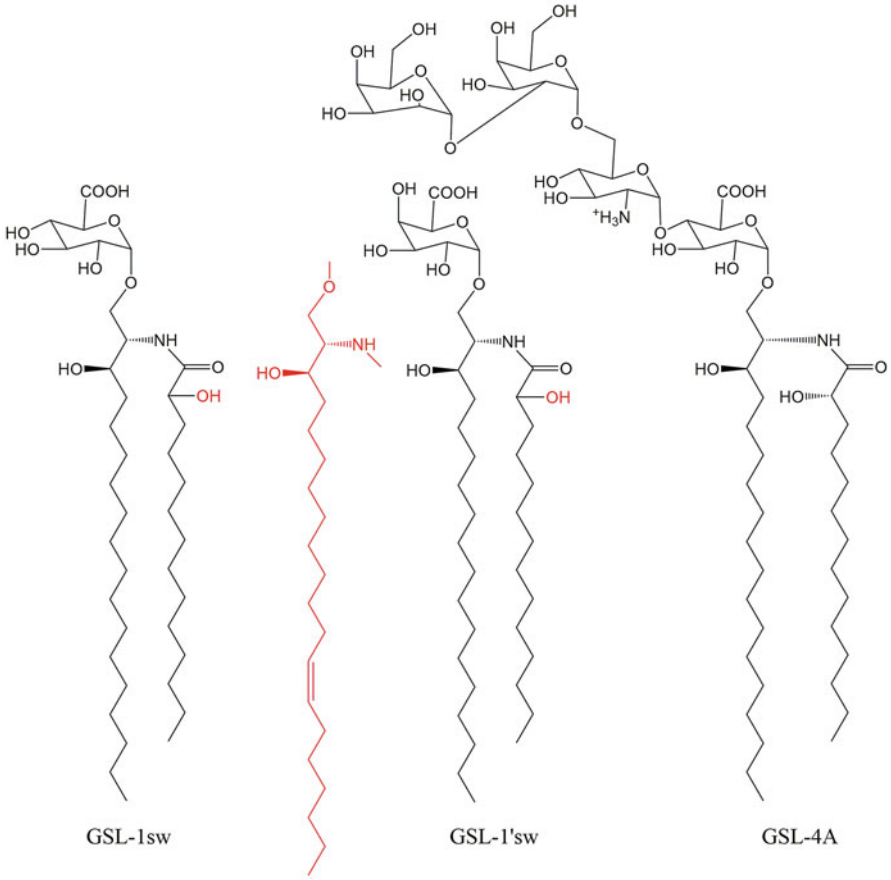

Fig. 1 Structures of sphingolipids GSL-1sw (α-glucuronosyl ceramide) and GSL-1′sw (α-galacturonosyl ceramide) from *Sphingomonas wittichii* (Kawahara et al. 2002) and GSL-4A from *Sphingomonas paucimobilis* (Kawahara et al. 1991). Structural variations in the amidified fatty acid (myristic acid or 2-OH-myristic acid) or in the dihydrosphingosine (2-amino-1,3-octadecandiol or 2-amino-*cis*-13,14-methylene-1,3-eicosandiol) residue of *S. wittichii* SphLs are highlighted

acyl-CoA to form 3-oxo-sphinganine (stage 1), followed by its reduction to sphinganine (stage 2), acylation to *N*-acylsphinganine (dihydroceramide) (stage 3), and desaturation to ceramide (stage 4) (Hanada 2003; Nieto et al. 2008). In stage 5, ceramide is modified with different polar groups to form the great diversity of sphingolipids. Although the eukaryotic genes involved in the sphingolipid biosynthetis are known (Kihara et al. 2007; Hirabayashi and Furuya 2008), little is known in bacteria. An exception is sphingolipid biosynthesis step 1 catalyzed by serine palmitoyltransferase (EC 2.3.1.50) (Fig. 2).

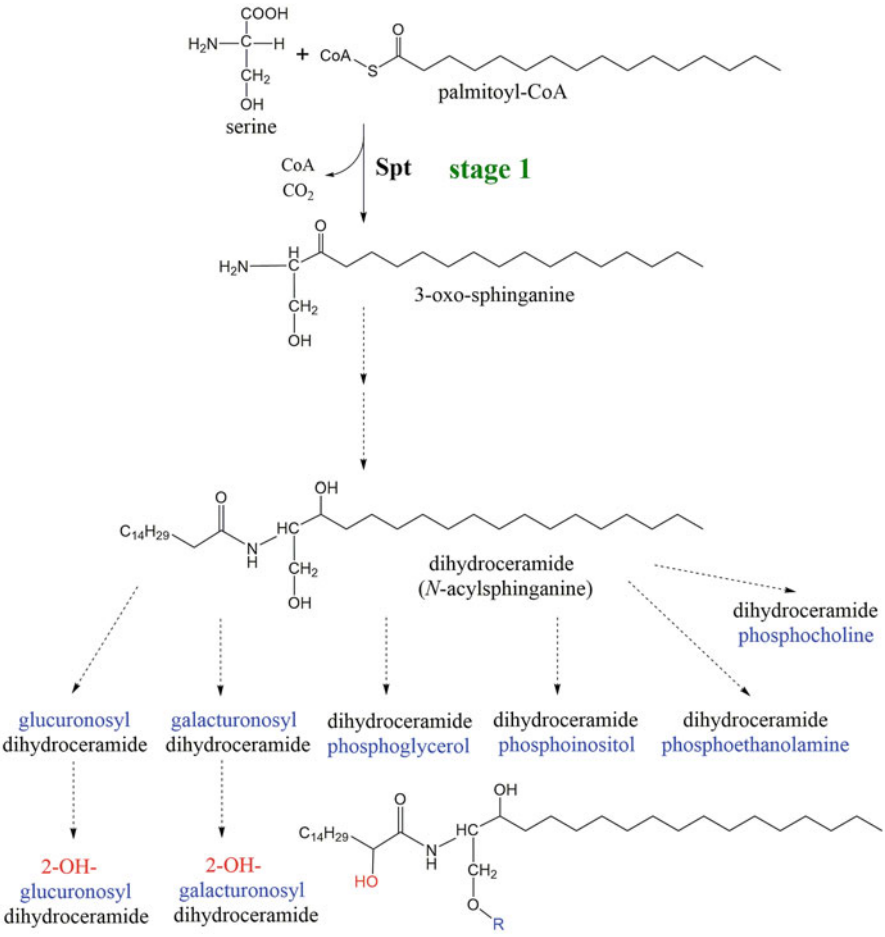

Fig. 2 Biosynthesis of sphingolipids in bacteria (see text for details). Head group modifications (R) and α-hydroxylation (2-OH) are highlighted

2.2.1 Bacterial Serine Palmitoyltransferase, an α-Oxoamine Synthase

Like other oxoamine synthases, the bacterial soluble serine palmitoyltransferase (Spt) is pyridoxal 5′- phosphate-dependent and performs a Claisen condensation between serine and the acyl-CoA thioester with a concomitant decarboxylation (Kerbarh et al. 2006). Although the Spt from *Sphingomonas* seems to be cytosolic, the Spts from *Sphingobacterium multivorum* and from *Bacteriovorax* (*Bdellovibrio*) *stolpii* are peripherically associated with the cytoplasmic side of the inner membrane (Ikushiro et al. 2001; Ikushiro et al. 2007). The *S. paucimobilis* Spt crystal structure (Yard et al. 2007) at 1.3 Å resolution shows that the enzyme is a symmetrical homodimer with two active sites composed of monomers, each consisting of three domains. The pyridoxal 5′-

phosphate cofactor is bound covalently to lysine 265 as an internal aldimine/Schiff base, and the active site is composed of residues from both subunits, located at the bottom of a deep cleft. Other common bacterial α-oxoamine synthases are 8-amino-7-oxononanoate synthase (BioF; EC 2.3.1.47), which catalyzes the formation of 8-amino-7-oxononanoate from 6-carboxyhexanoyl-CoA and L-alanine during biotin biosynthesis (Webster et al. 2000), 5-aminolevulinate synthase (HemA; EC 2.3.1.37), which catalyzes the formation of 5-aminolevulinate from succinyl-CoA and glycine during tetrapyrrole and heme biosynthesis in α-proteobacteria (Astner et al. 2005), and 2-amino-3-oxobutyrate coenzyme A ligase (Kbl; EC 2.3.1.29), which cleaves 2-amino-3-oxobutyrate into acetyl-CoA and glycine during threonine degradation (Kerbarh et al. 2006). Phylogenetic analysis of bacterial α-oxoamine synthases (Fig. 3) suggests that at least two distinct subgroups of bacterial Spts exist and that in one subgroup the encoding genes frequently form an operon with a putative acyl carrier protein gene. This finding suggests specialized acyl carrier proteins, instead of CoA, are used in some cases during the initial step of sphingolipid biosynthesis in bacteria (Geiger et al. 2010; Raman et al. 2010). Based on our analysis (Fig. 3), the ability to form SphLs is more widespread in α-proteobacteria (*Gluconobacter, Caulobacter*) than previously thought and might occur even in the β-proteobacterium *Nitrosomonas* and in several pathogenic *Escherichia coli* strains, i.e., in the enterotoxigenic *E. coli* (ETEC) B7A, but also in the host for protein expression *E. coli* BL21(DE3) (Jeong et al. 2015). Spts of the δ-proteobacteria *Bacteriovorax stolpii, Myxococcus xanthus*, or *Stigmatella aurantiaca* group with Spts of members of the *Bacteroidetes* (Wieland Brown et al. 2013) phylum (Fig. 3). Therefore, within the known bacterial phyla, genes for Spt have only been found in members of the *Proteobacteria* (α, β, γ, δ) or the *Bacteroidetes* which matches fairly well the reported occurrence of SphLs in these phyla. In eukaryotes, Spts are heterodimers consisting of distinct subunits (Ikushiro et al. 2007). All these eukaryotic subunits share a common origin (Ikushiro et al. 2007) (Fig. 3); however, they do not originate from within one of the known bacterial Spt clades (Fig. 3). Although recent work suggested that the homodimeric Spts (TgSPT1 and TgSPT2) of the eukaryotic protozoan parasite *Toxoplasma gondii* group more closely with bacterial Spts than with orthologues from animals, plants, or fungi (Mina et al. 2017), our phylogenetic tree (Fig. 3) does not support this idea. Therefore, a bacterial ancestor for eukaryotic Spts is presently not known.

2.2.2 Formation of Dihydroceramide

Although dihydroceramide is certainly formed in some bacteria, i.e., in *Acetobacter malorum* (Ogawa et al. 2010), *M. xanthus* (Lorenzen et al. 2014), or *Bacteroides fragilis* (Wieland Brown et al. 2013), it is not clear to date whether bacteria perform analogous steps as reported for stage 2 and 3 of eukaryotic sphingolipid biosynthesis in order to convert 3-oxo-sphinganine to dihydroceramide (Fig. 2).

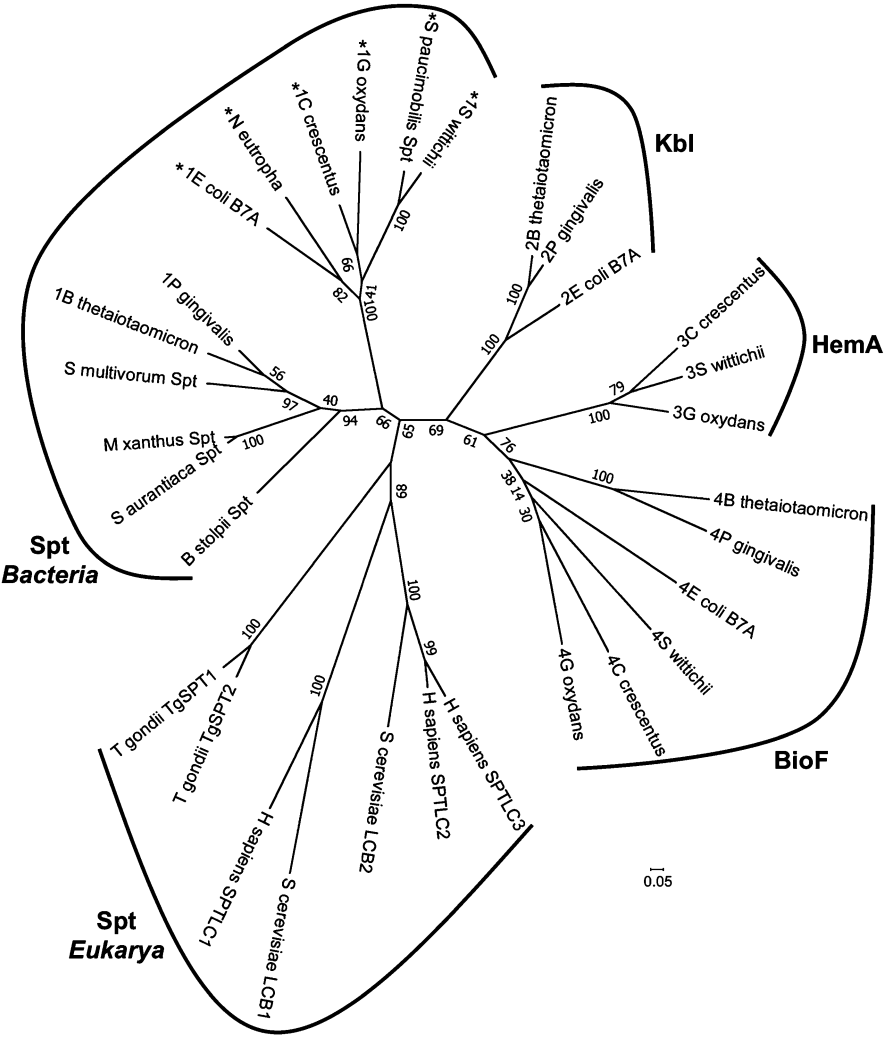

Fig. 3 Unrooted phylogenetic tree of selected bacterial and eukaryotic serine palmitoyltransferases (Spts) as well as other α-oxoamine synthases from bacteria. The amino acid sequences were aligned using the CLUSTALW program (http://www.expasy.ch/). The gap opening and extension parameters were set to 10 and 0.1, respectively. The tree was constructed using the program MEGA (http://www.megasoftware.net/) using the maximum likelihood method. Distances between sequences are expressed as 0.05 changes per amino acid residue. The number at each node represents the bootstrap value as a percentage of 500 replications. The asterisks label species in which the *spt* gene forms an operon with a putative structural gene for an acyl carrier protein. Accession numbers are as follows: *Bacteroides thetaiotaomicron* VPI-5482 (1: NP_809783; 2: NP_810284; 4: NP_810356), *Bacteriovorax stolpii* Spt (BAF73753), *Caulobacter crescentus* CB15 (1: NP_419978; 3: NP_420168; 4: NP_420387), *Escherichia coli* B7A (1: EDV60350; 2: ZP_03029941; 4: ZP_03030227), *Gluconobacter oxydans* 621H (1: AAW61792; 3: WP_011253160.1; 4: WP_011252295.1), *Homo sapiens* Spt subunits SPTLC1, SPTLC2, SPTLC3 (AAH68537, NP_004854, NP_060797), *Myxococcus xanthus* DK 1622 Spt

2.2.3 Diversification of Head Groups and α-Hydroxylation

Although it may be expected that dihydroceramide is the lipid anchor on which head group modification reactions can be performed (Fig. 2), in no case structural genes or enzymes involved have been reported in bacteria. The amidified fatty acyl residue in bacterial sphingolipids is often hydroxylated at the α- or 2-position. In the myxobacteria *Myxococcus xanthus* and *Stigmatella aurantiaca*, the fatty acid α-hydroxylases (Fah) have been identified (Ring et al. 2009) and it has been postulated that the intact sphingolipid is required in order to perform the hydroxylation (Fig. 2). Surprisingly, whereas the *S. aurantiaca* Fah forms the *R*-stereoisomer, the *M. xanthus* Fah synthesizes the *S*-isomer. Homologues of the *M. xanthus* Fah are encountered as well in genomes of the α-proteobacteria *Zymomonas mobilis* or *Gluconobacter oxydans* (Ring et al. 2009). Notably, the initially described bacterial SphLs, such as GSL-4A from *S. paucimobilis*, also display the hydroxylated *S*-isomer at the 2′-position (Kawahara et al. 1991) (Fig. 1).

2.3 Localization and Transport of Bacterial Sphingolipids

Over the last decade a number of essential components for the formation and transport of LPS have been identified in *Escherichia coli* (Whitfield and Trent 2014). The proteins involved in LPS transport (Lpt) form a complex, which bridges all the compartments of the cell reaching from the cytoplasm to the OM. Although most of the LPS biosynthesis takes place at the inner leaflet of the IM, the assembled LPS molecule is flipped to the outer leaflet of the IM by the MsbA flippase. In *E. coli*, seven proteins (LptABCDEFG) are known to be essential for cell viability and LPS transport from the outer leaflet of the IM to the outer leaflet of the OM. An ABC complex in the IM, consisting of the transmembrane proteins LptF and LptG and the ATP-binding LptB proteins, reclutes LPS from the outer leaflet of the IM. It is thought that subsequently, the LPS is transported across the periplasm through a channel formed by domains of the LptC, LptA, and LptD proteins. The carboxy-terminal domain of LptD forms a large β-barrel which is anchored in the OM and through which the LPS is moved to the outer leaflet of the outer membrane (Whitfield and Trent 2014).

It is remarkable that in SphL-producing bacteria such as *S. wittichii*, LptF and LptG homologues are encoded by genes localized in the neighborhood of SphL biosynthesis genes, such as the structural gene for Spt. In *S. wittichii*, these LptF and

Fig. 3 (continued) (ABF87747), *Nitrosomonas eutropha* C91 (WP_011633580), *Porphyromonas gingivalis* ATCC 33277 (1: WP_012458484; 2: WP_012458311; 4: WP_012457897), *Saccharomyces cerevisiae* Spt subunits LCB1, LCB2 (KZV09152, CAA98880), *Sphingobacterium multivorum* Spt (BAF73751), *Sphingomonas paucimobilis* Spt (BAB56013), *Sphingomonas wittichii* RW1 (1: WP_012050084; 3: WP_012050007; 4: WP_011952097), *Stigmatella aurantiaca* DW4/3-1 (EAU63634), and *Toxoplasma gondii* TgSPT1 (XP_002368482.1) and TgSPT2 (XP_002368481.1). For annotations of the distinct α-oxoamine synthases, see text.

LptG homologues are essential (Roggo et al. 2013). In contrast, bacteria that synthesize both LPS and SphLs have one set of genes coding for LptF and LptG homologues in the neighborhood of biosynthetic genes for LPS and another set in the neighborhood of SphL biosynthesis genes (data not shown). Therefore, it might well be that the principal transport machinery for moving LPS or SphLs from the inner surface of the IM to the outer surface of the OM is essentially the same, and that LptF/LptG homologues specific for LPS or other LptF/LptG homologues specific for SphLs would act as sorting machines and define whether LPS or SphLs are transported to the final destination in the outer leaflet of the OM.

2.4 Function of Bacterial Sphingolipids

In many Gram-negative bacteria that lack LPS in the outer leaflet of the outer membrane, SphLs seem to functionally replace them in this compartment, i.e., in *Sphingomonas* (Kawazaki et al. 1994) or *Sorangium* (Keck et al. 2011). In cases where bacteria can make both LPS and SphLs, a response to abiotic stress (acidity or elevated temperature) might be associated with an increase of SphLs or ceramide, i.e., in *A. malorum* (Ogawa et al. 2010).

In recent years, the α-proteobacterium *Caulobacter crescentus* has become one of the most-studied Gram-negative bacteria, as it serves as a model for asymmetric cell division and its regulation. Although *C. crescentus* has LPS, it also harbors genes that might be involved in the biosynthesis of SphLs (Geiger et al. 2010) (Fig. 3). In *C. crescentus*, presumptive genes for SphL biosynthesis or their transport to the OM are not essential (Christen et al. 2011); however, these genes are important for the "fitness" of the organism (Christen et al. 2011). As the phenotype of "fitness" is determined by growth of mutagenized bacterial populations to confluence and stationary phase on agar-containing solid culture media, "fitness" integrates many traits, representing a complex phenotype which is composed by several other more discernable phenotypes, i.e., affected growth, survival, competition, etc. Presently, the molecular basis for the "fitness" deficiency of potential SphL-lacking mutants is not clear.

The anaerobic *Bacteroides fragilis* is among the most abundant bacteria in the human digestive tract and is necessary as symbiotic microbe to maintain human health. Also, sphingolipids of *B. fragilis* are not required for exponential growth in culture media (An et al. 2014). However, they are essential for the survival of the bacteria in the mammalian intestine (An et al. 2011; Wieland Brown et al. 2013) and regulate the homeostasis of invariant natural killer T cells (iNKT)(An et al. 2014).

One of the mechanisms by which the innate immune system detects the invasion of pathogenic organisms is by means of Toll-like receptors (TLR) (Akira and Takeda 2004). Apparently, phosphorylated ceramides can be detected by the TLR2 receptor (Nichols et al. 2011), and α-galactosyl-ceramide is presented as lipidic antigen to iNKT cells by the CD1d protein which belongs to

the major nonpolymorphic histocompatibility complex, similar to class I (An et al. 2014). During endotoxin sensing and signaling, LPS seems to be recognized by the cluster of differentiation 14 (CD14), transferred to the myeloid differentiation factor 2 (MD-2), which in turn crosslinks TLR4 leading to a hexameric complex $(TLR4/MD\text{-}2/LPS)_2$ in the first step of the inflammatory process (Cochet and Peri 2017). However, ceramide also acts as a TLR4 agonist in a CD14-independent way (Fischer et al. 2007).

Although sphingolipids are not essential for the periodontal pathogen *Porphyromonas gingivalis*, they are important for survival in stationary phase of growth, for surviving oxidative stress, and for presenting the gingipain surface polysaccharides (Moye et al. 2016). *Porphyromonas gingivalis* phosphoglycerol dihydroceramide acts as a virulence factor enhancing osteoclastogenesis and a subsequent breakdown of bone tissue (Kanzaki et al. 2017).

During fruiting body formation in *M. xanthus*, ceramides accumulate (Arendt et al. 2015), but in spite of this correlation, the mechanistic details are not known.

In summary, bacterial sphingolipids might be essential in some cases (*Sphingomonas*, *Sorangium*), be important for survival in stationary phase of growth (*Bacteroides*, *Porphyromonas*), and provide resistance to heat (*Acetomonas*, *Bacteroides*), acid (*Acetomonas*), and oxidative stress (*Bacteroides*, *Porphyromonas*); however, molecular mechanisms are not known in any of these cases. There is clear evidence for the interaction of bacterial sphingolipids with eukaryotic signaling systems in some cases (*Sphingomonas*, *Bacteroides*), but bacterial sphingolipid signaling might be much more widespread than previously thought. The role of sphingolipids in host-microbial interactions has been reviewed recently (Heaver et al. 2018).

3 Sulfonolipids in the *Cytophaga* Group

Gram-negative bacteria of the *Cytophaga* group move by gliding and belong to the phylum *Bacteroidetes*. Major lipids in the membranes of *Cytophaga johnsonae* are sulfonolipids (SnoLs), ornithine-containing lipids (OL), and phosphatidylethanolamine (PE). SnoLs and OL are predominantly localized to the outer membrane, whereas PE is the predominant lipid of the inner membrane (Pitta et al. 1989). SnoLs contain capnine that is formed by the condensation of cysteate with fatty acyl-CoA under the release of CO_2 (White 1984; Abbanat et al. 1986) (Fig. 4a), in a reaction analogous to the one catalyzed by Spt (Yard et al. 2007). Capnine is then converted to *N*-acyl-capnine, the membrane-forming SnoL. The *N*-acylated residues vary between the *Cytophaga*, *Capnocytophaga*, and *Flexibacter* genera but include straight-chain and *iso*-fatty acids containing 14, 15, or 16 C-atoms, as well as 2-OH- (in *Flexibacter* only) and 3-OH-fatty acids (Godchaux and Leadbetter 1984). Mutants of *C. johnsonae*, deficient in gliding and SnoL biosynthesis, were

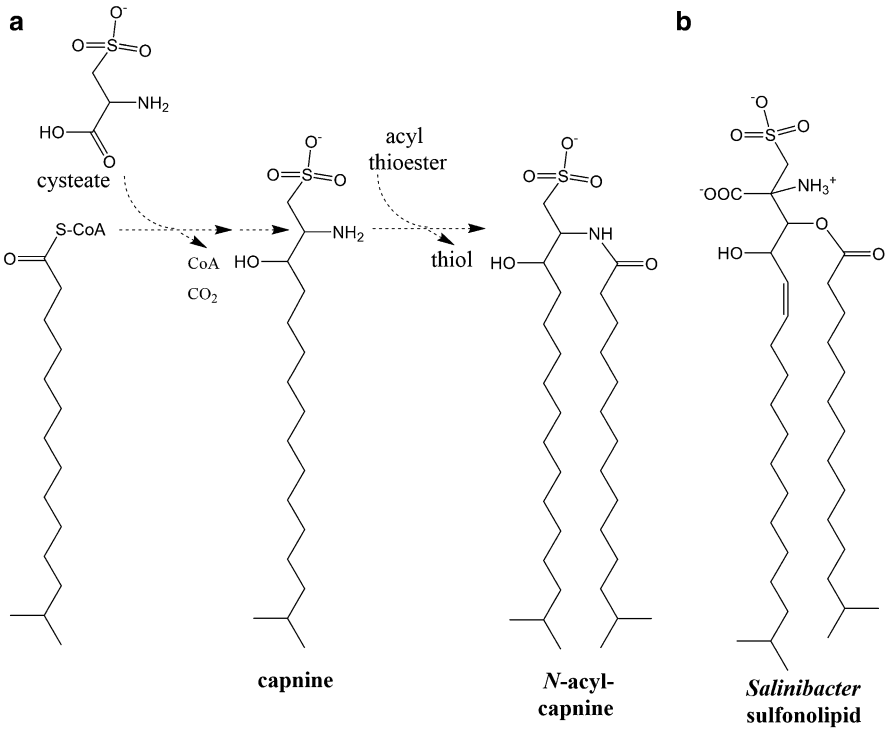

Fig. 4 Proposed pathway for sulfonolipid biosynthesis and an unusual sulfonolipid from *Salinibacter* (Reproduced from Geiger et al. 2010 with permission from Elsevier)

isolated and restoration of the SnoL content by providing cysteate resulted in recovery of the ability to glide (Abbanat et al. 1986). Therefore, SnoLs might be required for gliding motility. A structural variant of capnine exists in another member of the *Cytophaga* group, *Salinibacter ruber* (Corcelli et al. 2004). The *Salinibacter* sulfonolipid contains an extra carboxylate at carbon 2 and an *O*-acyl group at carbon 3 (Fig. 4b) that is diagnostic for this extremely halophilic bacterial genus (Corcelli et al. 2004).

In choanoflagellates, the closest living relatives of animals, multicellular rosette development might represent an initial step towards the evolution of multicellularity in animals. Formation of rosette colonies is regulated by environmental bacteria. The rosette-inducing bacterium *Algoriphagus machipongonensis* produces SnoLs termed rosette-inducing factors (Rif-1, Rif-2) (Fig. 5) which trigger the development of multicellular rosettes by the choanoflagellate *Salpingoeca rosetta* at femtomolar concentrations (Alegado et al. 2012). However, for full bioactivity, also lysophosphatidylethanolamines from *Algoriphagus* are required to synergize with rosette-inducing factors for rosette induction (Woznica et al. 2016). Notably, other *Algoriphagus* SnoLs are inactive for rosette formation, whereas the capnine IOR-1 functions as an inhibitor of rosettes (Woznica et al. 2016).

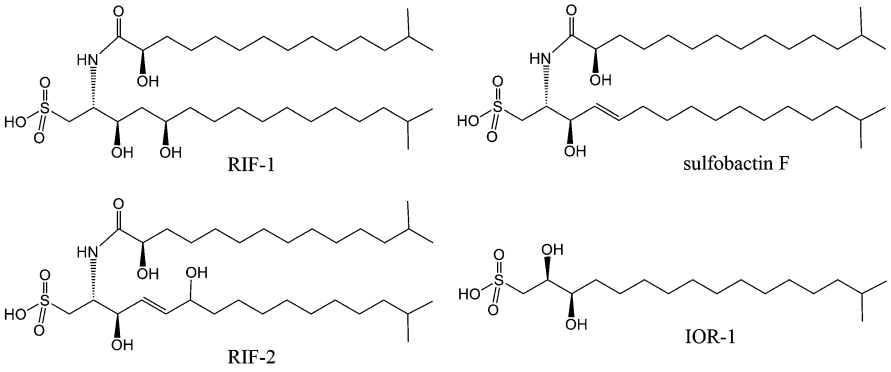

Fig. 5 Structures of sulfonolipids termed rosette-inducing factors (Rif-1, Rif-2), of an inactive sulfonolipid (sulfobactin F), and the IOR-1 capnine inhibitor of rosette formation.

4 Conclusions and Research Needs

Although sphingolipids (SphLs) are considered to be typical eukaryotic lipids, many more SphL-containing bacteria have been characterized in recent years. In bacteria, they seem to be localized to the outer leaflet of the outer membrane and in eukaryotes they seem to be enriched in the outer layer of the plasma membrane. A comparison of SphL biosynthesis enzymes is presently only possible with Spts. However, to date, it is not clear from which prokaryont the eukaryotes might have inherited the SphL biosynthetic machinery and for now the evolutionary history of SphLs remains a mystery.

So far most steps (including the corresponding structural genes and enzymes) in bacterial SphL or SnoL biosyntheses are not known, and it is unclear how SphLs or SnoLs are transported from their presumed site of biosynthesis (inner leaflet of the IM) to their presumed final destination (outer leaflet of the OM).

The functional implications of bacterial SphLs are only starting to emerge, but evidence suggests that they have important roles in protecting the bacterial producer as well as for the interaction with the abiotic and biotic environment.

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