

REVIEW ARTICLE

Sphingolipids controlling ciliary and microvillar function

 Fabian Kaiser, Mylene Huebecker and Dagmar Wachten 

Institute of Innate Immunity, Biophysical Imaging, Medical Faculty, University of Bonn, Germany

Correspondence

M. Huebecker and D. Wachten, Institute of Innate Immunity, Biophysical Imaging, Medical Faculty, University of Bonn, 53127 Bonn, Germany
 Tel: +49-2288-9656-311
 E-mails: mylene.huebecker@uni-bonn.de (M. H.); dwachten@uni-bonn.de (D. W.)

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Cilia and microvilli are membrane protrusions that extend from the surface of many different mammalian cell types. Motile cilia or flagella are only found on specialized cells, where they control cell movement or the generation of fluid flow, whereas immotile primary cilia protrude from the surface of almost every mammalian cell to detect and transduce extracellular signals. Despite these differences, all cilia consist of a microtubule core called the axoneme. Microvilli instead contain bundled linear actin filaments and are mainly localized on epithelial cells, where they modulate the absorption of nutrients. Cilia and microvilli constitute subcellular compartments with distinctive lipid and protein repertoires and specialized functions. Here, we summarize the role of sphingolipids in defining the identity and controlling the function of cilia and microvilli in mammalian cells.

Keywords: cilia; flagella; glycosphingolipids; microvilli; sphingolipids

The intracellular space is divided into subcellular compartments, containing a molecular architecture that is distinct from the rest of the cell. Cilia and microvilli are membrane protrusions that emanate from the surface of a variety of different cell types and constitute unique subcellular compartments with specialized function. Cilia come in two different flavors: Motile cilia move extracellular fluids or propel cells for active movement, whereas primary, nonmotile cilia function as cellular antenna that detect extracellular cues and locally transduce this information into a cellular response [1]. Prominent examples of motile cilia are cilia on lung epithelial cells that clear the lung of mucus, or flagella on sperm cells or flagellates. Primary

cilia can be found on almost every eukaryotic cell, where they are thought to fulfill sensory functions. Microvilli are membrane protrusions that increase the cell surface exchange area while minimizing any increase in volume. Therefore, they control a number of cellular processes, for example, absorption, secretion, cell adhesion, or mechanotransduction. The core structure of a cilium is the microtubule-based axoneme. Motile cilia contain nine outer microtubule doublets and one central microtubule pair, which is missing in the axoneme of primary cilia [2] (Fig. 1). In contrast, the structural core of microvilli consists of a dense bundle of cross-linked actin filaments [3] (Fig. 1).

Abbreviations

acyl-CoA, acyl-coenzyme A; aPKC, atypical protein kinase C; aSMase, acid sphingomyelinase; BBM, brush border membrane; BLM, basolateral membrane; Cer, ceramide; CERT, ceramide-transfer protein; CHO, Chinese hamster ovary; DHCer, dihydroceramide; ER, endoplasmic reticulum; ETEC, enterotoxigenic *Escherichia coli*; FAPP2, Phosphatidylinositol 4-Phosphate Adaptor Protein-2; FGA1, $\alpha(1-2)$ fucosyl GA1 glycosphingolipid; GalCer, galactosylceramide; GBA2, beta-glucosidase 2; GCS, glucosylceramide synthase; GlcCer, glucosylceramide; GPCR, G protein-coupled receptor; GSK3, glycogen synthase-3; GSL, glycosphingolipid; Hh, Hedgehog; Laurdan, 2-dimethylamino-6-lauroyl-naphthalene; MDCK, Madine-Darby Canine Kidney; MLR, Membrane Lipid Replacement; Na-Pi cotransporter, sodium gradient-dependent phosphate transporter; NPC1, Niemann-Pick disease type C1; nSMase-2, neutral sphingomyelinase-2; PKD, polycystic kidney disease; PUFA, polyunsaturated fatty acids; RTK, receptor tyrosine kinase; SM, sphingomyelin; SPA, sphinganine; SPO, sphingosine; TGN, trans-Golgi network; TRI/II, transforming growth factor- receptors I and II; VLCFA, very long-chain fatty acids; VLCPUFA, very long-chain polyunsaturated fatty acids; WNT, Wingless Int-1.

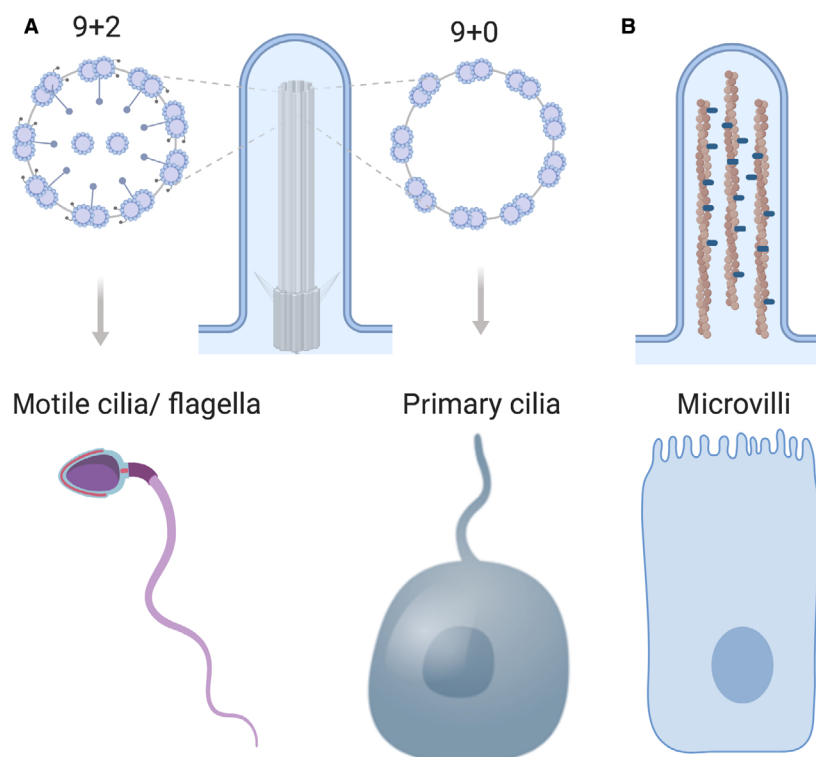


Fig. 1. Cilia and microvilli. (A) Cilia. The hallmark of the cilium is the microtubule-based axoneme, which consists of nine outer and one inner microtubule doublet for motile cilia, or only nine outer microtubule doubles for immotile primary cilia. (B) Microvilli. The microvillar core consists of bundled linear actin filaments with their plus ends facing the tip.

Subcellular compartments contain a unique protein and lipid composition that contributes to their function. The protein composition of cilia or microvilli has been studied in detail. However, information regarding the lipid composition of these compartments is lacking behind. Sphingolipids, in particular glycosphingolipids (GSLs), have been proposed to play important roles in determining the identity and function of subcellular compartments.

Glycosphingolipids are sphingolipids that have a diverse and large family of glycans attached to a ceramide (Cer) backbone, which is composed of the long-chain amino alcohol sphingosine (SPO) in amide linkage to a fatty acid [4]. GSL biosynthesis occurs in a stepwise fashion and starts in the endoplasmic reticulum (ER) with a sphingoid base from either *de novo*-synthesized sphinganine (SPA) or from the salvage pathway-derived SPO and acyl-coenzyme A (acyl-CoA). Depending on the substrate, ceramide synthases catalyze the reactions to produce either Cer or dihydroceramide (DHCer) from SPO or SPA, respectively [5]. In the ER lumen, Cer is either galactosylated to produce galactosylceramide (GalCer) or picked up at the ER membrane for transport to the Golgi complex by two different mechanisms [6,7]. On the one hand, the ceramide-transfer protein (CERT) transports Cer to the cis-Golgi network, where it mainly forms

sphingomyelin (SM) after translocation across the bilayer. On the other hand, after vesicular transport of Cer to the cis-Golgi, glucosylation of Cer in the cytosolic leaflet forms glucosylceramide (GlcCer) [8,9]. GlcCer builds the core structure of most mammalian GSLs and is either translocated to the luminal side for vesicular transport, or transported by nonvesicular transport via FAPP2 (Phosphatidylinositol 4-Phosphate Adaptor Protein-2) to the trans-Golgi. Glycosylation of GlcCer by the Golgi glycosyltransferases generates more complex GSL species and is dependent on the localization of the corresponding enzymes and the path the GSL was transported [10]. In general, vesicular transport vehiculates both GSLs and SM from the trans-Golgi network (TGN) to the plasma membrane, where they mainly reside in the extracellular leaflet, due to their orientation in the TGN [11]. Cell surface-specific glycosidases can partially remodel GSLs at the plasma membrane, and SM can also be further metabolized there [12,13]. GSLs and SM are constantly turned over and endocytosed from the plasma membrane for degradation in late endosomes and lysosomes [11]. Similar to their biosynthesis, GSL degradation occurs stepwise by glycohydrolases, which cleave the glycan moieties until only Cer is left, while SM is degraded to Cer by acid sphingomyelinase (aSMase) [14]. Finally, acid ceramidase breaks down

Cer into the fatty acid and sphingosine, which can be returned to the ER and again used for GSL biosynthesis from the salvage pathway [15]. An overview is shown in Fig. 2. Of note, understanding the distribution of each of these lipids (luminal or cytosolic) within the membrane of the residence organelle(s) is crucial to clarify GSL and sphingolipid function. A detailed overview of these features can be found here [16].

Glycosphingolipids are ubiquitously present in the membranes of eukaryotic cells, where they are essential for a variety of biological cell functions. For example, GSLs are involved in cell adhesion and migration, cell signaling, cell proliferation, endocytosis, intracellular transport, inflammation, and apoptosis [9,17]. Thus, GSL functions are mediated by both trans interactions (e.g., cell–cell interactions via binding to complementary molecules on opposing plasma membranes) and lateral, cis interactions (e.g., modulating activities of ion channels and receptors in the same plasma membrane) [18].

A tightly regulated and unique lipid and protein composition is central for ciliary function [19,20]. The apical membrane of polarized cells, for example, epithelial cells, is enriched in cholesterol and GSLs [21,22]. FAPP2 mediates the transport of GlcCer to the trans-Golgi and fosters synthesis of complex GSLs, especially globosides, at the Golgi complex. FAPP2 seems to be crucial for maintaining the unique lipid identity of the apical membrane, where primary cilia form, by conveying lipids and maintaining cellular polarization [23]. Elegant microscopy studies identified a small area around the cilium, the periciliary membrane, also called ciliary pocket when invaginating into the cytoplasm [24,25]. Given that GSL and cholesterol contents crucially determine order of cellular membranes, it is remarkable that laurdan (*2-dimethylamino-6-lauroyl-naphthalene*) microscopy revealed differences in membrane order in and around the cilium. Specifically, a zone at the base of the cilium displayed a higher membrane order compared with the surrounding apical and ciliary membrane [23]. At the most distal part of the ciliary membrane, freeze–fracture electron microscopy identified a structure termed the ‘ciliary necklace’ [26]. At least for motile ependymal cilia, it has been shown that this region is low in cholesterol [27]. Thus, the ciliary compartment contains lipid subdomains that might be important for ciliary function. Of note, a similar concept has been shown for the distribution of phosphoinositides: The phosphoinositide-regulating enzymes OCRL, INPP5b, and INPP5E, which all use phosphatidylinositol(4,5)-bisphosphate as a substrate, display distinct locations

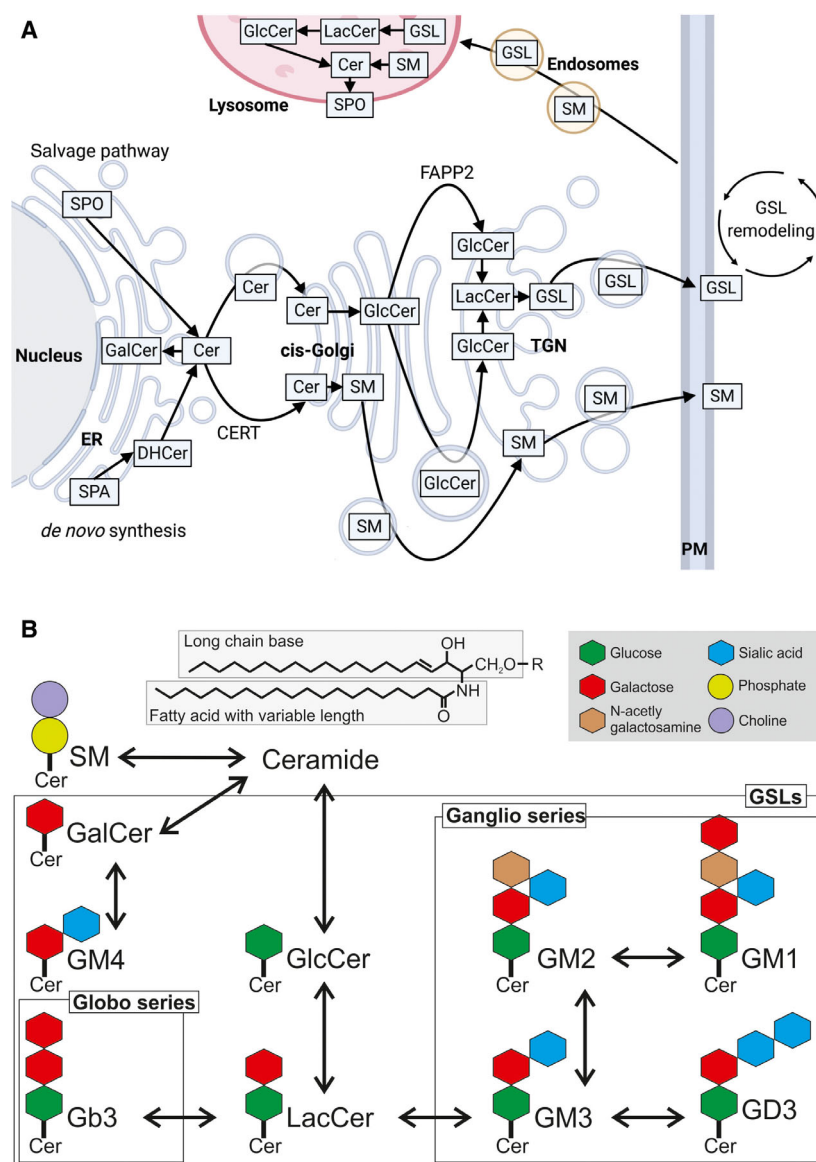
in the ciliary membrane [28]. Furthermore, the gangliosides GM1 and GM3 have been identified in primary cilia of epithelial Madine–Darby Canine Kidney (MDCK) cells. However, it is not known whether they reside inside the same subdomains or are part of two distinct entities [29]. Moreover, prominin-1, which is not only a cholesterol-dependent lipid-raft marker, but also contains a ganglioside-binding motif, is present in the ciliary membrane and might therefore determine the localization of gangliosides in different membrane domains [30,31]. Cer has also been proposed to be an integral part of the primary cilium in MDCK cells [32]. Ciliary G protein-coupled receptors (GPCR) and Wntless Int-1 (WNT) signaling seem to be regulated by the ciliary GSL content; however, except GM1 and GM3 in MDCK cells, the GSL species in the cilium have not been determined yet [33,34]. Most recently, it has been demonstrated that the ciliary membrane contains both ‘accessible’ and ‘inaccessible’ cholesterol, the latter complexed by sphingomyelin [35]. In summary, the molecular mechanisms that regulate the ciliary sphingolipid composition are still ill-defined.

Sphingolipids in primary ciliary biology

Sphingolipids in ciliogenesis

The cilium is a dynamic structure, closely linked to the cell cycle [36]. Ciliogenesis starts in the interphase, while the cilium is disassembled throughout G1 and S phases of the cell cycle [25]. Different modes of ciliogenesis have been proposed, depending on the cell type [37]. Thus, sphingolipids might regulate ciliogenesis in one, but exhibit a different role in other cell types. In epithelial cells, the basal body docks to the apical membrane and the cilium emanates, leading to local plasma membrane remodeling into the ciliary membrane [24]. In fibroblasts or smooth muscle cells, a membrane vesicle, termed primary vesicle, engulfs the distal end of the mother centriole, which converts into the basal body and further matures and migrates until it docks to a distinct place at the plasma membrane. The plasma membrane fuses with the ciliary vesicle, which then becomes the ciliary and periciliary membrane [38]. How the ciliary membrane is generated remains elusive. A growing number of studies have shown that sphingolipids, especially Cer, play a pivotal role in ciliogenesis. Ciliogenesis is impaired in FAPP2-deficient MDCK cells [23]. Furthermore, in MDCK cells, aSMase degrades apical SM to Cer [39,40]. Cer clusters in the apical ceramide-enriched compartment, where it activates atypical protein kinase C (aPKC) to

Fig. 2. Overview sphingolipid metabolism. (A) Glycosphingolipids (GSLs) and sphingomyelin (SM) are synthesized in the endoplasmic reticulum (ER) and Golgi complex (Golgi) and can partially be remodeled at the plasma membrane (PM). Turnover predominantly takes place in the endosomal and lysosomal compartments. It is important to note that to understand GSL and sphingolipid function, knowledge of the organelle membrane topology for each lipid, that is, luminal or cytosolic side, is crucial. A detailed overview about these features can be found here [16]. TGN, trans-Golgi network. SPO, sphingosine. SPA, sphinganine. DHCer, dihydroceramide. Cer, ceramide. GalCer, galactosylceramide. CERT, ceramide-transfer protein. GlcCer, glucosylceramide. LacCer, lactosylceramide. SM, sphingomyelin. FAPP2, Phosphatidylinositol 4-Phosphate Adaptor Protein-2. (B) Sphingolipid structures of the major lipid species described in this review.



drive ciliogenesis by preventing HDAC6 activation [40]. This mechanism also occurs in human embryonic stem cells and pluripotent stem cell-derived neuroprogenitors [32]. Here, in contrast to MDCK cells, neutral sphingomyelinase-2 (nSMase2) has been demonstrated to be essential for the conversion of SM to Cer. In particular, nSMase2-derived very long-chain C24:1 Cer forms attachment sites at the apicolateral cell membrane of neuroprogenitors to sequester aPKC, thereby reducing Aurora A kinase and HDAC6 activation and promoting ciliogenesis. Along this line, later studies identified an evolutionary-conserved mechanism of ceramide-mediated translocation of glycogen synthase kinase-3 (GSK3) into *Chlamydomonas* flagella and

ependymal cilia, which is critical for ciliogenesis [41]. An overview about the role of sphingolipids in primary ciliary biology is shown in Fig. 3.

Role of sphingolipids in ciliary signaling under physiological and pathological conditions

While the ciliary proteome under physiological and pathological conditions has been extensively studied, the contribution of ciliary membrane lipids has only recently drawn attention [20,42]. Here, several groups elegantly unraveled the molecular bases of phosphatidylinositol-dependent ciliary signaling [25,43,44]. Still, the regulation of fundamental ciliary signaling

pathways, for example, Hedgehog (Hh) signaling, signaling through a variety of GPCRs, Wntless Int-1 (WNT) signaling, and receptor tyrosine kinase (RTK) signaling, including PDGFR α , insulin, IGF1, EGFR, and TGF- β /BMP by lipids is largely unknown [45]. Sphingolipids are known to regulate many of these pathways [33,34,46–48]. However, whether the regulation occurs in the cilium is only known for a few signaling pathways. For example, a ceramide-dependent mechanism that suppresses cell migration and tumor metastasis by restricting the transforming growth factor- β receptors I and II (T β RI/II)-Hh signaling selectively at the plasma membrane of the primary cilium has been recently identified [49]. Interestingly, the neurodegenerative lysosomal storage disorder Niemann–Pick disease type C1 (NPC1) has been linked to ciliary function. Here, the accumulation and mislocalization of unesterified cholesterol, SPO, and sphingolipids seems to reduce primary cilium length and dysregulate Hh signaling in hippocampal neurons [50]. In addition, an NPC1 mouse model showed altered dopamine

transporter expression and differences in primary ciliary number and length–frequency distribution in the dorsal striatum [51]. There is also experimental evidence for the presence and interaction between SM and cholesterol in the ciliary membrane. Here, SM regulates the levels of accessible cholesterol and therefore Hh signaling [35]. Furthermore, in an unbiased screen, ceramide synthase 5 and 6 (*Cers5*, *Cers6*), serine palmitoyltransferase long-chain base subunit 1 and 2 (*Sptlc1*, *Sptlc2*), sphingosine-1-phosphate phosphatase (*Sgpp1*), Sphingomyelin synthase 1 (*Sgms1*), and delta (4)-desaturase sphingolipid 2 (*Degs2*) have been identified as regulators of Hh signaling [35]. Future work has to demonstrate the role of these proteins in regulating the ciliary sphingolipid homeostasis.

Ciliary dysfunction leads to severe diseases commonly referred to as ciliopathies [52]. One of the most common ciliopathies is the polycystic kidney disease (PKD). GlcCer and GM3 levels are increased in the kidney of PKD models [53]. Block of glucosylceramide synthase (GCS) or knockout of GM3 synthase in

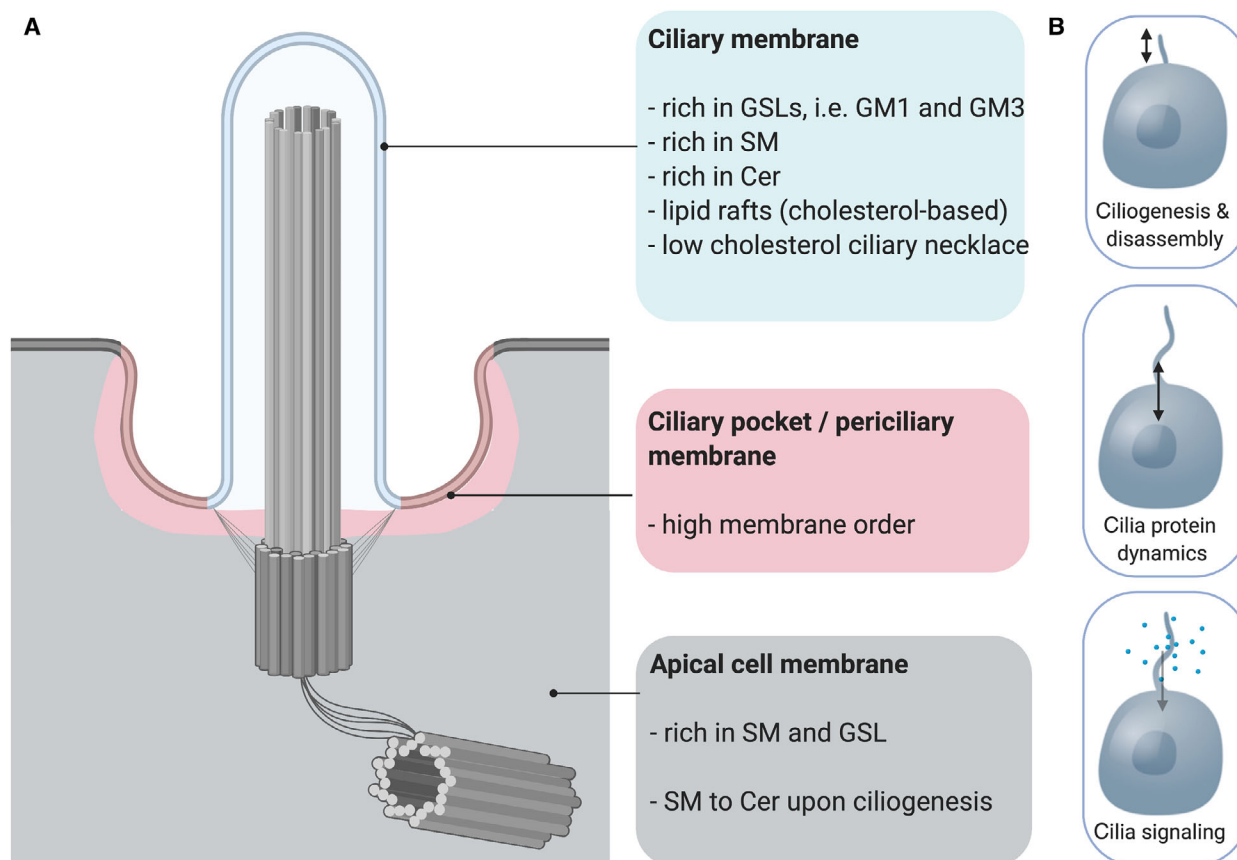


Fig. 3. Sphingolipids in cilia. (A) Schematic representation of a cilium. Known and predominant lipid species in the apical, periciliary, and the ciliary membrane are indicated. (B) Cilia functions controlled by sphingolipids.

PKD mouse models reduced cystogenesis and diminished pathological AKT-mTOR signaling [53,54]. Most products of PKD-associated genes are expressed in primary cilia or centrosomes [55–57]. Moreover, GM1 and GM3 are present in the ciliary membrane of MDCK cells and are known to regulate RTK signaling [29,46]. Thus, dysregulation of ciliary sphingolipids might play a role in the development of PKD.

In summary, cilia seem to regulate signaling and function by changing lipid content and dynamics. Analysis of the ciliary lipidome in combination with high-resolution imaging studies focusing on the interplay between ciliary signaling and sphingolipid content and distribution will improve our understanding of how sphingolipids regulate ciliary function in physiological and pathophysiological conditions.

Sphingolipids in flagellar biology

Sphingolipid composition of flagella

The protein composition of flagella has been studied in detail and revealed a great level of complexity, with more than 500 different proteins being identified and many proteins being highly conserved between eukaryotes [58]. However, the function of lipids and proteins in subcellular compartments is closely intertwined and information about the lipid composition of flagella is lacking behind. Most of the information regarding the lipid and protein composition of flagella comes not only from unicellular organisms, such as *Chlamydomonas*, *Paramecium*, *Tetrahymena*, and *Trypanosoma* [59], but also from other cell types containing a single flagellum, that is, sperm. *Trypanosoma brucei* synthesizes certain sphingophospholipid classes in a life cycle-dependent manner [60]. Furthermore, parasites in the procyclic bloodstream form (the proliferative stage found in the midgut of the insect vector, the tsetse fly) contain GSLs preferentially in the flagellum [61]. Early studies in *Paramecium* indicated that flagella are enriched in raft-type lipids, that is, sphingophospholipids [62]. This has been supported by studies in *Trypanosoma brucei* using Laurdan microscopy, demonstrating that lipids in the flagellar membrane are in a more ordered state than in the cell body membrane [61]. The plasma membrane of sperm flagella shows species-specific differences and also displays some distinct features compared with other membranes. Early reports demonstrated that sphingolipids are the major class of neutral glycolipids, which are predominantly of the globoseries [63–65]. Sphingomyelin makes up 10–15% of the total lipids in sperm [66]. Sphingomyelins and ceramides in mammalian

sperm contain nonhydroxylated polyenoic very long-chain fatty acids (VLCFA, > 22 carbon atoms) [67–70] and 2-hydroxylated polyenoic VLCFA [67,71]. A spatial comparison between sperm head and flagellum revealed that the head contained sphingomyelin species with nonhydroxy- and 2-OH very long-chain polyunsaturated fatty acids (VLCPUFA), and the tail contained most of the saturated fatty acids that are present in the total sphingomyelins from sperm [72]. In contrast, the abundant ceramide species, predominantly made up by species with 2-OH VLCPUFA, were only located in the tail [72] (Fig. 4).

Role of sphingolipids in controlling flagellar functions

To fertilize the egg, sperm need to mature and undergo a process called capacitation [73,74]. Loss of sperm sterols is an early step in capacitation [75]. The major sterol in sperm is cholesterol, which inhibits capacitation [76]. It has been demonstrated that sphingomyelin slows down cholesterol depletion and therefore the rate of capacitation [77]. In turn, exogenous addition of sphingomyelinase accelerates capacitation by promoting cholesterol depletion and by generating ceramide [77]. In line with this finding, capacitation increases the ceramide pool by hydrolyzing sphingomyelins, mainly the species containing VLCFA [78]. Ceramide has been shown to enhance acrosomal exocytosis, which is needed to penetrate and fertilize the egg [79] (Fig. 4). The acrosome is a Golgi-derived organelle that develops over the anterior half of the sperm head and contains enzymes that break down the outer membrane of the oocyte, the zona pellucida. Acrosomal exocytosis is also controlled by sphingosine 1-phosphate (S1P) [80,81]. Human sperm have been proposed to produce S1P when experiencing an exocytic stimulus, which evokes acrosomal exocytosis in a G_i protein-coupled manner [80]. In addition, GSLs seem to migrate into the sperm head upon capacitation [82].

Not only sperm maturation, but also sperm viability and motility are controlled by sphingolipids. Inhibition of ceramide synthesis using the mycotoxin fumonisin B1, which increases the concentration of sphingoid bases (SPA, SPA, and their phosphorylated derivatives) and depletes levels of ceramide and complex sphingolipids, reduces sperm motility and suppresses sperm production [83,84]. Membrane integrity is essential to maintain sperm viability and motility. Sperm are highly susceptible to oxidative stress, as they are rich in sensitive polyunsaturated fatty acids (PUFA), and are unable to synthesize and repair many essential membrane constituents. Membrane

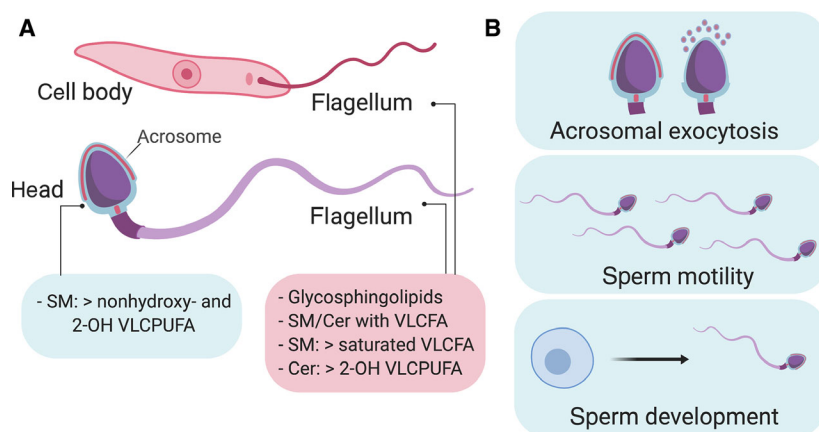


Fig. 4. Sphingolipids in flagella. (A) Schematic representation of a *Trypanosoma* and a human sperm. The preferential lipid distribution in the sperm head and the flagellum is indicated. (B) Sperm functions controlled by sphingolipids.

Lipid Replacement (MLR) with glycerophospholipid mixtures improves sperm viability, motility, and resistance to oxidizing agents, suggesting that MLR may be used to treat infertility [85]. In addition, not only the function of sperm flagella, but also the function of ependymal motile cilia and flagella in *Chlamydomonas* is controlled by sphingolipids. Both express GSK3, which is critical for ciliogenesis. GSK3 is activated by (phyto)ceramide, and inhibition of (phyto)ceramide synthesis causes flagellar loss and a defect in ciliary motility [41].

Role of sphingolipids in controlling sperm development

Sphingolipids do not only regulate flagellar function, but also regulate flagellar development. This has been well documented for the development of sperm flagella. Here, the GSL GlcCer seems to play an important role (Fig. 4). The GlcCer homeostasis during sperm development is predominantly controlled by the beta-glucosidase 2 (GBA2) [86]. Loss or inhibition of GBA2 causes accumulation of GlcCer and, in turn, male subfertility due to severe sperm morphological defects, in particular, of the sperm head [86–88]. Of note, levels of ceramide or sphingomyelin remain unchanged [87]. GlcCer accumulation in GBA2 knockout mice alters cytoskeletal dynamics in both germ cells and Sertoli cells due to a more ordered lipid organization in the plasma membrane. In particular, actin polymerization and therefore the organization of F-actin structures in the ectoplasmic specialization between germ and Sertoli cells and of microtubules in the sperm manchette were affected [87]. Furthermore, microtubule polymerization was also facilitated. Both cytoskeletal structures have been shown to be crucial for shaping the sperm head and forming the acrosome

[89], which explains why GBA2 knockout mice display severe sperm head defects [86,87].

Sphingolipids in microvilli biology

Microvilli are cellular membrane protrusions found on the apical plasma membrane of polarized epithelial cells. The tightly packed, microvilli-covered epithelial surface is known as brush border or brush border membrane (BBM). The brush border is a complex and highly plastic subcellular compartment that increases the cellular surface area, facilitating absorption and secretion of nutrients and ions [90]. Accordingly, BBMs are found in the intestinal tract, the kidney, and the liver.

Sphingolipids in the brush border membrane

The polarized phenotype of epithelial cells is maintained by the segregation and retention of specific proteins and lipids in distinct apical and basolateral plasma membrane domains [91]. Similar to cilia and flagella, the lipid composition of microvilli has not been extensively studied. However, an asymmetric distribution of lipids between apical brush border membranes (BBMs) and basolateral membranes (BLMs), which are separated by tight junctions, is well established [80,92,93] (Fig. 5). Of note, these findings predominantly derive from studies using kidney and intestinal epithelium. In rat renal proximal tubule epithelial cells, a distinct distribution of GSLs between BBM and BLM has been observed, that is, an enrichment of specific gangliosides in the BBM (GM3, GM1, and GD1a) vs. the BLM (GM4 and GD3) [80]. However, the distribution of neutral GSLs, that is, GlcCer and Gb3, is similar between both membranes [80]. The GSL composition of the small intestine epithelium in

humans is also distinctly different to nonepithelial residue, showing a fivefold higher GSL content [94]. Interestingly, gangliosides inserted into the apical BBM are unable to diffuse past the tight junctions to the BLM, suggesting the existence of a specific sorting process for epithelial GSLs [95]. Furthermore, using nanoscale imaging techniques, it has been demonstrated that gangliosides distribute not only in an apical–basolateral manner, but also in a more microscopic peak–valley manner, implicating a unique distribution of GM1 and GM3 on the apical membrane of polarized cells [96].

Cholesterol in the brush border membrane

The BBM of epithelial cells, for example, intestinal enterocytes, renal cells, and placental syncytiotrophoblasts, is characterized by a high cholesterol content. Several decades ago, it had already been proposed that cholesterol in the BBM is present in at least two states, a smaller readily accessible pool and a larger pool, which may interact with membrane proteins [97]. Nowadays, using high-resolution imaging techniques, it has been shown that accessible cholesterol is indeed not evenly distributed over the entire plasma membrane, but instead is highly enriched, that is, on microvilli of Chinese hamster ovary (CHO) cells [98].

Lipid rafts in the brush border membrane

Glycosphingolipids and cholesterol are characteristic of lipid rafts. Lipid rafts are nanoscale GSL/cholesterol-enriched membrane microdomains, which function as membrane sorting and signaling platforms [99]. Indeed, lipid rafts are also present in the BBM, for example, of pig enterocytes, and are highly enriched in

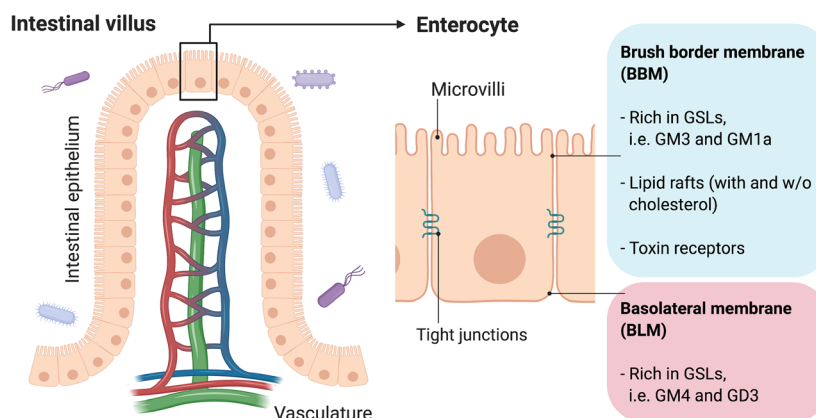
GSLs ([100]; reviewed in [101]). The abundance of lipid rafts in the BBM is further supported by proteomic analysis of apical microvillar membranes of human syncytiotrophoblast cells, which contain a protein composition that is highly reminiscent of the composition in lipid rafts [102]. It has been suggested that lipid rafts in BBMs of enterocytes and kidney cells come in different flavors: cholesterol-dependent and more stable cholesterol-independent rafts [100,103,104]. The cholesterol-binding protein prominin-1, a specific marker of plasma membrane protrusions like microvilli, has been used to demonstrate coexistence of different cholesterol-based lipid rafts within the apical membrane [105,106]. Here, proteins targeted to the apical BBM of microvilli reside in spatially segregated subdomains (i.e., microvillar and planar subdomains), suggesting the coexistence of multiple distinct types of cholesterol-based raft-like assemblies of lipids and proteins in the apical plasma membrane [106]. Besides cholesterol, GSLs also contribute to the segregation into membrane domains: GM1 colocalizes with prominin-1 on microvilli, whereas GM3 is segregated from this domain, suggesting that it is localized in the planar region [29]. Functionally, prominin-1/ganglioside membrane complexes have been shown to organize the architecture and regulate the dynamics of microvilli in MDCK cells via an interplay with phosphoinositide 3-kinase and cytoskeleton components [107].

Sphingolipids and microvillar functions

Within microvilli, sphingolipids control essential cellular functions such as morphology, stability, and differentiation, as well as specialized cellular functions such as protein sorting and signaling within lipid rafts.

Fig. 5. Sphingolipids in microvilli.

Schematic representation of an intestinal villus, focusing on the intestinal epithelium with the brush border. A magnified view of the organization of a single enterocyte with microvilli is shown to the right. The polarized phenotype of an enterocyte features the distinctive apical brush border membrane (BBM) and the basolateral membrane (BLM), which are separated by tight junctions. The preferential lipid distribution in the BBM and the BLM is indicated.



Sphingolipids in control of microvillar morphology and stability

In general, lipid rafts, consisting of sphingolipids and cholesterol, play an important role in microvillar maintenance and formation. Inhibition of either sphingolipid or cholesterol biosynthesis reduced the number of microvilli on the surface of MDCK cells [108]. Microvillar formation has also been abolished by a reduction in ganglioside GM3 synthesis (using the mycotoxin fumonisin B1), suggesting that constant sphingolipid synthesis is required for the assembly and maintenance of this subcellular compartment [109]. Furthermore, the interaction of sphingomyelin and cytoskeletal proteins has been shown to be essential for the formation of microvilli [110]. GSLs also modulate membrane properties: The high glycolipid content of the BBM results in a lower lipid fluidity compared with other mammalian plasma membranes [111].

Sphingolipids in microvillar protein sorting and targeting (lipid rafts)

The absorption of dietary lipids and nutrients in the intestine and the absorption and secretion of ions in the kidney are key functions of the BBM. To fulfill this function, microvilli require a sophisticated targeting mechanism for lipids and membrane proteins (e.g., enzymes or transporters), which distinguish between the BBM and BLM. However, the underlying molecular mechanisms are not well understood [112]. Sphingolipids play an important role in membrane protein targeting and function, and some examples will be discussed in the following section.

It is known for decades that changes in ganglioside composition of the apical membrane alter sodium transport across kidney epithelial membranes [113]. Sphingomyelin and cholesterol also distinctly modulate sodium-coupled uptake in renal proximal tubular cells [114]. The glucocorticoid dexamethasone modulates phosphate transport of the renal BBM by increasing the GlcCer content, which selectively modulates Na/Pi cotransport and sodium gradient-dependent phosphate transporter (Na-Pi cotransporter) abundance [115]. Thus, renal Na/Pi cotransport can in part be regulated through alterations in BBM lipid composition, including SPO, GlcCer, and ganglioside GM3 content, and a decrease in BBM lipid fluidity [116]. Segregation of the Na/Pi cotransporter into the GSL-enriched apical membrane domains modulates diffusion, clustering, and activity of the protein [117].

Kidneys are particularly rich in the GSL Gb3, but its physiological function remains ill-defined. A recent study has suggested that Gb3 in the BBM of the renal proximal tubule facilitates the absorption of albumin and pharmacological inhibition of GlcCer-derived GSL synthesis, including Gb3, protects against acute kidney injury [118].

In the intestinal BBM, digestive enzymes like the sucrase-isomaltase, which is required to digest dietary carbohydrates, are sorted to the apical membrane through post-translational modification and interaction with sphingolipid-cholesterol microdomains [119]. The intestinal degradation of dietary sphingolipids is also regulated by the neutral ceramidase, encoded by the *Asah2* gene, which localizes to the BBM [120]. Ceramidases are key enzymes in the regulation of Cer, SPO, and sphingosine 1-phosphate levels. The neutral ceramidase is highly expressed in the BBM of the small intestine, indicating that it is key for the catabolism of dietary sphingolipids and regulates the levels of bioactive sphingolipid metabolites in the intestinal tract [120].

Studies using mice with enterocyte-specific genetic deletion of the *Ugcg* gene, encoding the enzyme UDP-glucose ceramide glucosyltransferase that catalyzes the initial step of GSL biosynthesis, revealed that GSLs are essential for the resorptive function of enterocytes via endocytosis and vesicular transport, but not for cell polarization [120].

In summary, sphingolipids play an important role not only in targeting receptors and enzymes to the BBM, but also in modulating their functions post-translationally.

Sphingolipids in cochlear hairs cells

Gangliosides are enriched in the central nervous system, and ganglioside deficiencies have been shown to cause various neurological disorders. This is particularly true for patients carrying mutations in the *ST3GAL5* gene, encoding GM3 synthase [121–123]. Mice lacking GM3 synthase are devoid of GM3 and display severe hearing loss accompanied by degeneration of cochlear hair cells [124]. Cochlear hair cells possess stereocilia (or stereovilli), which are non-motile apical protrusions closely related to microvilli and are important for signal generation in the auditory system. Further studies in human and mice demonstrated that GM3 is essential for the development and structural integrity of stereocilia on cochlear hair cells and therefore auditory function [125].

Microvillar sphingolipids as receptors for toxins

Bacteria cause a huge variety of diseases in animals and humans. To this end, bacteria have evolved distinct strategies to adhere to specific cellular targets and to enter the host cell through hijacking the cellular machinery. Due to their cell surface location and variability and abundance in membranes, GSLs and glycoproteins provide a wide range of binding sites for bacteria, toxins, and more generally lectins [126]. Here, we will focus on interactions of bacterial toxins with GSLs in the BBM of the intestinal tract.

Cholera toxin

The ganglioside GM1 has been identified as the receptor for cholera toxin in intestinal epithelial brush borders of various species, including human small intestine [127]. Cholera toxin is a protein complex secreted by the bacterium *Vibrio cholera* and is responsible for the massive, watery diarrhea characteristic of cholera infection. The B subunit of cholera toxin binds to GM1 gangliosides on the surface of target cells, followed by endocytosis of the entire toxin complex [127–129].

Enterotoxins

While cholera toxin predominantly binds to the ganglioside GM1 in intestinal epithelial BBM, *Escherichia coli* heat-labile enterotoxin binds to both GM1 ganglioside and glycoprotein receptor sites of the small intestinal epithelium [130,131]. Enterotoxins are produced by enterotoxigenic *Escherichia coli* (ETEC) and are one of the leading bacterial causes of diarrhea. Heat-labile enterotoxins work similar to Cholera toxins, as the B subunit binds to its GSL and glycoprotein receptors on the surface of target cells, followed by endocytosis of the entire toxin complex.

Shiga toxin

The Shiga toxin works in a slightly different way. Shiga toxin mostly derives from the bacteria *Shigella dysenteriae* and some serotypes of *Escherichia coli*. The B subunits of the toxin bind to the GSL Gb3 in the BBM [132,133]. Binding of the subunit B to Gb3 induces the formation of narrow tubular membrane invaginations and, in turn, formation of inward membrane tubules for its cellular uptake [134].

Toxins as research tools

Due to the high receptor specificity, the toxins are powerful tools to detect and modulate specific sphingolipid species. For example, fluorophore conjugates of the B subunit of cholera toxin are regularly used to label neurons or lipid rafts that are rich in GM1 [135,136]. However, the labeling has to be interpreted with caution as cholera toxin binding is not exclusive for GM1, but it also binds GM2 and GD1b, or even glycoproteins [130,137–139].

Microbiome and sphingolipids

The microbiome in the intestine profoundly affects the host physiology and vice versa, intestinal conditions affect the composition and metabolism of the microbiome. When comparing the glycolipid content of the microvillar membrane in the small intestine between germ-free mice (without a microbiome in the intestine) and conventional animals, a clear difference was observed: conventional mice contained higher levels of α (1-2) fucosyl GA1 glycosphingolipid (FGA1), containing C18-phytosphingosine as long-chain base and 2-hydroxy fatty acids [140,141]. The physiological function of FGA1 remains, however, unknown. Recent studies have also demonstrated that *Bacteroidetes* from the gut microbiome produce α -galactosylceramide (α GalCer). In contrast, germ-free mice are devoid of (α GalCer) [142]. Western diet, colitis, or an influenza A infection decreased α GalCer levels, suggesting that α GalCer is produced by commensals in the mouse intestine and that the stressful conditions causing dysbiosis alter its synthesis [142].

Conclusions and future perspectives

In summary, sphingolipids have been shown to be important for ciliary and microvillar function. They determine the unique lipid identity of these subcellular compartments, but also fulfill sensory functions by acting as receptors or even as messenger molecules. We are just about to understand these functions under physiological conditions. Future studies will reveal how dysregulation of sphingolipid homeostasis in subcellular compartments like cilia and microvilli contributes to cellular dysfunction and therefore disease development. The recent advance in mass spectrometry imaging may help shed light on how sphingolipids are organized in subcellular compartments and how this, in turn, determines specific cellular and physiological functions.

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