Minireview

Role of sphingomyelinase and ceramide in modulating rafts: do biophysical properties determine biologic outcome?

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Abstract **Recent biophysical data suggest that the properties of ceramide observed in model membranes may apply to biological systems. In particular, the ability of ceramide to form microdomains, which coalesce into larger platforms or macrodomains, appears to be important for some cellular signaling processes. Several laboratories have now demonstrated similar reorganization of plasma membrane sphingolipid rafts, via ceramide generation, into macrodomains. This event appeared necessary for signaling upon activation of a specific set of cell surface receptors. In this article, we review the properties and functions of rafts, and the role of sphingomyelinase and ceramide in the biogenesis and re-modeling of these rafts. As clustering of some cell surface receptors in these domains may be critical for signal transduction, we propose a new model for transmembrane signal transmission.**

Key words: Raft; Ceramide; Sphingomyelinase; Capping

1. Introduction

The following review focuses on the mechanisms by which ceramide can serve its second messenger role, exploring the links between biophysical and biological data. Ceramide has been recognized as a key signaling molecule, shown to mediate cellular functions as diverse as apoptosis, cell cycle arrest, differentiation, and senescence. However, the exact mechanism(s) by which ceramide performs its functions are not completely understood. Recent biophysical data, as well as the increasing body of work implicating ceramide signaling in membrane rafts or microdomains, have provided clues to show that changes in membrane structure are critical to ceramide function. In this article, we review the properties and functions of membrane rafts, and the role of sphingolipids, especially sphingomyelin and ceramide, as well as sphingomyelinase, in the biogenesis and maintenance of these microdomains. How the generation of ceramide from sphingomyelin alters the dynamics of rafts and in turn affects various signaling processes is discussed. In particular, this review focuses on the unique biophysical properties of ceramide to induce membrane fusion, pore formation and the generation of microdomains. An attempt is made to relate these properties to its biological functions, in particular to the reorganization of raft microdomains into ceramide-enriched patches, which then coalesce into larger platforms. As clustering of some cell surface receptors in these macrodomains appears critical for their signal transduction, these studies describe a new mode of transmembrane signal transmission.

2. Ceramide: biophysical properties and biological effects

A body of literature has appeared in the past decade that has changed the perception of the plasma membrane. For many years, the Singer–Nicholson model of the cell membrane was the accepted one: it views the membrane as a 'fluid mosaic' in which a homogeneous phospholipid bilayer acts mainly as a solvent for integral proteins and as a permeability barrier for the cell. However, the perception of the cell membrane structure and dynamics has changed in the last decade with the introduction of concepts such as lateral heterogeneity, lipid microdomains, and rafts. The plasma membrane is now viewed as a heterogeneous dynamic entity, containing various lipid domains that can assume different functional states.

Ceramide is a sphingolipid that has gained much attention as an important signaling molecule in vital cell processes as diverse as apoptosis, growth arrest, senescence, differentiation, mediating an immune response, and cell cycle arrest [1–3]. Structurally, ceramides are composed of a fatty acid chain, of varied length, saturation, and hydroxylation, bound via an amide linkage to the amino group of a sphingoid base. Ceramides constitute the hydrophobic backbone of all the complex sphingolipids: sphingomyelin, cerebrosides, gangliosides, and others. The fatty acid chain length of ceramide can vary from two to 28 carbons, although C-16 to C-24 ceramides are most abundant in mammalian cells. These fatty acids are usually saturated or monounsaturated, and sometimes may contain a hydroxyl group at the C-2 position (α-hydroxy fatty acid) or on the terminal C atom (ω-hydroxy fatty acid) [1,2]. Ceramides are among the least polar, most hydrophobic lipids in nature. This might explain in part their abundance in the stratum corneum, the barrier preventing water evaporation through the skin. Sphingomyelin is composed of a ceramide backbone with a phosphorylcholine headgroup attached to the C-1 free alcohol group of ceramide.

Ceramide can be generated within a cell either through a de novo synthesis pathway mediated by ceramide synthase, or

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through hydrolysis of cellular sphingomyelin via an acid or neutral sphingomyelinase [1]. There is evidence to suggest that both these pathways are involved in ceramide generation in response to different stimuli. Both acid and neutral sphingomyelinases have been identified, differing in their ion dependence, pH optimum, or cellular localization [5]. A full description of the stimuli and sphingomyelinase enzymes that regulate ceramide production is beyond the scope of this review (for further information see [2,3]).

Several downstream targets for ceramide action have been identified, and clues as to the mechanism by which ceramide might be mediating its diverse cellular effects are beginning to emerge. Recent data from a number of laboratories have begun to suggest that some of the effects of ceramide are mediated by its unique biophysical properties. The polar headgroup of ceramide, the amide linkage, as well as the hydroxyl groups of sphingosine and the fatty acid chain, confer onto ceramide the capacity to form extensive hydrogen bonds in the phospholipid bilayer. The tight interactions between ceramide molecules give rise to in-plane phase separation of ceramide-rich and -poor microdomains [4]. This propensity to undergo extensive hydrogen-bonding is what differentiates sphingolipids from glycerophospholipids. While the latter can only act as acceptors of hydrogen bonds, sphingolipids, such as ceramide and sphingomyelin, can act as both acceptors and donors through their hydroxyl and amide groups. However, ceramides and sphingomyelin differ in their affinity for other lipids in the membrane, namely cholesterol. Sphingomyelin interacts very tightly with cholesterol, through hydrogen-bonding between the C-3 hydroxyl group of cholesterol and the sphingosine moiety of sphingomyelin, and this serves as the basis for raft formation, as discussed in more detail below. Ceramides, on the other hand, have very poor affinity for cholesterol and tend to separate into exclusive ceramide-enriched microdomains [4]. Furthermore, as a hydrophobic molecule, ceramide strongly favors partitioning into bilayers, thus implying the possibility that changes in the physical state of the membrane might play a role in ceramide-mediated function. A better understanding of the properties of ceramide-containing model membranes has been provided by a number of biophysical research groups over the past few years. These data have been invaluable in understanding the lipid–lipid and lipid–protein interactions that may be determining the activities of ceramide in biologic membranes.

Ceramides mix poorly with phospholipids in bilayers, segregate into distinct high-temperature melting ceramide-enriched microdomains, and facilitate the lamellar–hexagonal transition of lipids [6]. These properties affect the ordering of lipids in the membrane, tending to destabilize them and cause efflux, fusion, or budding of vesicles. The recent literature has suggested that membrane fusion is critical for many cellular processes such as membrane biogenesis and viral infections [4]. In this review, we focus on three properties of ceramide that mediate its functions: its ability to induce membrane fusion/fission, pore formation, and lateral separation into microdomains.

One of the effects of ceramide when added to model membranes is its ability to induce membrane fusion or fission. Ruiz-Arguello et al. [7,8] showed that treatment of large unilamellar vesicles containing sphingomyelin, phosphatidylethanolamine, and cholesterol with sphingomyelinase generated ceramide within seconds, accompanied by vesicle aggregation or efflux of intravesicular components. Moreover, addition of ceramide at 5–10 mol% in phosphatidylcholine (PC)-containing vesicles reduced the time required for fusion of smaller vesicles generated by the treatment of these PC-containing vesicles with phospholipase C. The effect of sphingomyelinase was due to the production of ceramide which itself promoted the lamellar to non-lamellar phase transition and induced fusion. Montes et al. [9] showed that in large unilamellar vesicles composed of different phospholipids and cholesterol, both externally added and enzymatically produced ceramide (10 mol% of total lipid) induced release of vesicle contents. Furthermore, Holopainen et al. [10] showed microscopic images of rapid ceramide-enriched microdomain formation within 30 s of sphingomyelinase treatment of PC/sphingomyelin large unilamellar vesicles, followed by vesicular budding. These biophysical findings may have a biologic correlate. Zhang et al. [16] observed that endocytic vesicles, 400 nm in diameter, are formed in ATP-depleted macrophages when treated with exogenous sphingomyelinase or ceramide. These vesicles are free of caveolin or clathrin, and pinch off the plasma membrane to become internalized. This is in agreement with the biophysical data that show that while sphingomyelin promotes the stability of lipid bilayers, ceramide, due to its relatively small polar headgroup (conical shape), induces a negative curvature, favoring vesicle formation.

Ceramide has also been shown to induce pore formation. Siskind and Colombini [11] showed that C-2 and C-16 ceramide have the capacity to form pores in model phospholipid bilayers whereas the channel-forming capacity of dihydroceramide is limited. This is an interesting finding since in mammalian cells, dihydroceramide is also inactive [1]. The authors argue that the pore-forming ability is due to extensive hydrogen-bonding capacity of ceramide, which is greatly reduced in the dihydroceramide molecule. This might provide one mechanism to explain some of the biological effects of ceramide: after ceramide generation at the plasma membrane in response to stress- or agonist-induced receptor aggregation, a local alteration in the plasma membrane permeability barrier might result in abnormal ion fluxes. Release of ions, such as calcium or others, would in turn affect the activity of local enzymes, activating specific local signaling cascades.

One of the effects of ceramide when added to model membranes is its ability to induce lateral segregation followed by microdomain formation. Huang et al. [13] examined the structure of bilayers composed of deuterated dipalmitoyl PC and bovine brain ceramide using nuclear magnetic resonance spectroscopy. They observed that the addition of ceramide induced lateral phase separation of fluid phospholipid bilayers into regions of gel, and liquid crystalline phases, where ceramide partitioned mostly into the gel phase. Similarly, Veiga et al. [6] showed that in the lamellar phase, ceramides do not mix ideally with phospholipids, giving rise to the co-existence of domains that undergo gel–fluid transition at different temperatures. Ceramide-enriched microdomains were also detected in PC/phosphatidylserine mixtures using pyrene-labeled fluorescent phospholipids to probe lateral mobility in the membrane [15]. In these studies, atomic force microscopy revealed that long chain ceramides mixed very poorly with cholesterol and lateral phase separation ensued [15], indicating that ceramide possesses the ability to spontaneously form microdomains in a glycerophospholipid bilayer. These investiga-

tions in model membranes are reminiscent of biologic membranes. It is now well known that lipids are distributed non-randomly in the plasma membrane leading to the formation of sub-microscopic microdomains termed rafts. Rafts are composed mainly of sphingomyelin and cholesterol [14] (for more detail, see Section 3), and comprise gel-like regions of tightly packed sphingolipids, compared to the rest of the liquid crystalline phospholipid bilayer.

All of this helps us understand the mechanisms by which ceramide might signal: the generation of ceramide in response to a stress signal or other agonist induces local changes in the membrane environment. This in turn could affect the permeability and fluidity of the membrane, induce ion fluxes, increase movement of proteins into or from rafts, cause conformational changes in membrane-associated enzymes or receptors, as well as alter the transbilayer movement of lipids. The poor miscibility of ceramides and phospholipids and the subsequent formation of ceramide-rich membrane domains might also cause packing defects, allowing ceramide to modulate membrane-bound enzyme activity in this way. Huang et al. [13] suggested this to be the mechanism of activation of phospholipase A_2. Ceramide generation might also change membrane curvature, altering the three-dimensional structure of enzymes residing in the membrane, either activating or inhibiting them. A direct binding site for ceramide on certain proteins is also possible, in a manner similar to diacylglycerol binding to protein kinase C (PKC). One such paper postulates that ceramide binds to the cysteine-rich domains of kinase suppressor of ras (KSR) [12]. This concept is discussed further below. In Section 3, we will explore in more detail the role of ceramide in lipid rafts.

3. Membrane rafts

With the discovery of membrane rafts, the plasma membrane is now viewed as a heterogeneous dynamic entity, organized into compositionally and functionally specific domains which are not static, but are rather in equilibrium with the rest of the membrane lipids. Rafts are lateral assemblies of sphingolipids and cholesterol that form from the tight hydrophobic interactions between these molecules. In this model, sphingolipids associate laterally with one another through weak interactions between the carbohydrate heads of the glycosphingolipids and the hydrophobic interaction between their saturated side chains. Any voids between associated sphingolipids are filled by cholesterol molecules which also interact with the hydrophobic portion of sphingolipids [17]. The tight interactions between cholesterol and sphingomyelin in the membrane, as described in Section 2, is the driving force that segregates them from the rest of the plasma membrane phospholipids that remain more fluid in nature [18]. Lipid analysis has revealed that up to 70% of total cellular sphingomyelin is found in rafts [23]. Treatment of cells with cholesterol-depleting agents leads to disruption of rafts by sequestering cholesterol and removing it from the membrane, and is a technique commonly used to study the function of these rafts.

There are two types of rafts: those containing the structural protein caveolin-1 that form caveolae, and those that lack this protein but express two different raft-specific proteins, called flotillin-1 and 2 [19]. As caveolar and non-caveolar rafts are highly enriched in sphingolipids and glycosphingolipids, they are also known as glycolipid-enriched microdomains. These rafts are also highly enriched in gangliosides, especially GM_1 which has almost exclusively been localized to them. GM_1 is the cellular cholera toxin receptor, and the toxin's affinity for it is often used as a marker for these microdomains. Rafts are resistant to solubilization with cold non-ionic detergents, whereas the rest of the membrane lipids are soluble [20]. This biochemical property has been the basis of a technique that separates membrane rafts from the rest of the plasma membrane on sucrose density flotation gradients. For this reason, rafts have also been termed detergent-insoluble glycolipid-enriched microdomains.

Non-caveolar microdomains can be 10–300 nm in size and can assume different shapes, but are flat rather than invaginated [21]. When the structural protein caveolin-1 is inserted into them, they form the invaginated 50–100 nm flask-shaped structures termed caveolae. Caveolae were first discovered in the late 1950s by Palade and Yamada [22], who at the time observed these small flask-shaped structures in the membrane that looked like 'little caves', and hence gave them the name caveolae [23]. Since Palade observed these structures in endothelial cells, he proposed that they are involved in the uptake of materials from the blood and their transport across the cells, a process referred to as transcytosis. For decades, the exact function of caveolae remained obscure. However, in the early 1990s, and with the discovery of integral structural protein caveolin-1, scientists developed methods that allowed them to isolate these structures and subsequently analyze their composition and function. Caveolae have been found in several cell types, including fibroblasts, adipocytes, endothelial and epithelial cells, and smooth muscle and striated muscle cells, whereas non-caveolar rafts are believed to be ubiquitously present in cells. Caveolins are palmitoylated hairpin-like proteins and they include caveolins-1 and 2, which are usually co-expressed in a variety of cells, and caveolin-3 which is restricted to muscle cells.

4. Functions of rafts

It is now known that the composition and function of membrane rafts can be modulated in response to a variety of factors and stress conditions, and thus a correlation between the physical state of the membrane and the physiological state of the cell can be envisioned.

Many well known signaling proteins have been shown to either reside in or be transferred into or out of rafts during the process of signal transmission. These include the epidermal growth factor (EGF) receptor, insulin receptor, non-receptor tyrosine kinases (fyn and src), G proteins, Ras, nitric oxide synthase (NOS), adenylate cyclase, PI3 kinase, several PKC isoforms, Fas, and the tumor necrosis factor-α (TNF) receptor [23]. The list of raft-associated proteins is increasing rapidly.

Caveolin-1 can directly interact with many of these signaling molecules via a conserved 20 amino acid domain termed the caveolin scaffolding domain, which appears to hold molecular targets in an inactive conformation. The best characterized example of this interaction is the association of caveolin-1 with endothelial NOS (eNOS) [23,24]. eNOS is found in endothelial cells and produces NO in response to various stimuli including hormones and neurotransmitters. NO helps dilate and relax the blood vessels allowing for easier blood

flow. Several groups have now shown that eNOS is bound to caveolin-1, and that this interaction keeps it inactive [25], whereas signals that activate eNOS induce its dissociation from caveolin-1 [26]. Injection of mice with a peptide that mimics caveolin-1 prevents NO production by sequestering eNOS, and reduces inflammation of the ears of mice treated with mustard oil [26].

Similarly, the EGF receptor interacts with caveolin-1 within caveolae of quiescent fibroblasts, and rapidly exits in response to EGF stimulation [27]. Migration out of caveolae appears to be important for normal cellular function since mutant EGF receptors, which are incapable of moving out of caveolae, induce an oncogenic phenotype [27].

Alternatively, some proteins migrate into caveolae, which in some instances may be a ceramide-regulated event. Giaccia and co-workers showed that radiation-induced ceramide generation modulated caveolin-1 function, leading to inhibition of phosphoinositide 3′-kinase (PI3K) that had been translocated into caveolae. This effect was mimicked by the addition of C-2 ceramide exogenously [28]. Migration into rafts has also been observed with CD-40 and the Fas receptor, for which ceramide plays a role in signal transmission, and will be addressed subsequently in this review [29,47].

5. Capping: definition and the role of rafts in this process

Capping is a process during which cell surface receptors or proteins aggregate on one pole of the cell after binding their cognate ligands or agonistic antibodies [30]. This is believed to be a prerequisite for signaling by many receptors such as the insulin, L-selectin, EGF, Fas, and immunoglobulins, to list a few [30]. For instance, the release of histamine from mast cells is known to result from the rapid clustering of Fc receptors of IgE on the cell surface.

The exact mechanism of the capping process is still poorly understood, but it is believed that the cytoskeleton is either directly or indirectly involved in the lateral redistribution of surface molecules into a cap structure. The involvement of contractile microtubules was reported many years ago. Wessels et al. [31] observed an effect of cytochalasin B in partially inhibiting capping in mouse splenic lymphocytes. However, in other cell lines, cytochalasin B promoted capping. This suggests that differences exist in the way microfilaments are involved in capping in different cells, and for different receptors. The literature suggests at least two mechanisms for capping: one which involves transmembrane linkage between surface receptors and the sub-membrane microfilament and cytoskeletal network [30], and another which relies more on the properties of membrane microdomains to direct the interaction with receptors [29,32].

The role of microdomains in capping is evolving. Evidence for the role of lipids and microdomains in modulating the capping process came long before the concept of membrane rafts emerged, through the work of Hoover et al. [33]. Their studies showed that as the cellular levels of cholesterol decreased, capping of surface immunoglobulins in murine lymphocytes was reduced as well. The authors proposed that the capped proteins were found in a gel-like part of the membrane, and that removal of cholesterol reduced that gel state, increased fluidity and thus interfered with capping [33]. It is now well established that membrane rafts are indeed in a gel-like state. The importance of sphingolipid-enriched rafts in receptor capping also came from the work of Macphee and Barker [34]. These investigators showed that extended exposure to C-2 ceramide resulted in trkA activation by enhancing formation of trkA dimers, through effects that involve changes in plasmalemmal lipid composition and properties of rafts. Concomitantly, it was shown that CD28 engagement on the surface of T lymphocytes leads to clustering of rafts at the site of T cell receptor (TCR) engagement, and the formation of a dense cap [35,36]. It was proposed that this process might serve to amplify and sustain TCR-induced signaling, resulting in increased recruitment of activating kinases to rafts and segregation of phosphorylated substrates from phosphatases [37]. Direct evidence supporting this notion was provided by Janes et al. [41] who showed that aggregation of lck and the TCR by anti-CD-3 antibodies occurred within rafts, while CD-45, a protein tyrosine phosphatase which regulates lck activity, was found outside rafts [42]. It has even been shown by Harder et al. that direct cross-linking of raft-associated ganglioside GM_1 by cholera toxin results in co-capping of GM_1, Thy 1, the TCR complex, and the src tyrosine kinase fyn, in a signaling cluster [39]. Furthermore, capping can occur in non-caveolar rafts. Stuermer et al. demonstrated that flotillin-1 and 2 associate with the activated TCR complex during capping [40] in neurons and Jurkat cells that do not express caveolin-1 and lack caveolae. Activation of some non-receptor protein tyrosine kinases and a myriad of receptors also seem dependent on association with rafts [43,44]. The importance of sphingolipid rafts in capping is further emphasized by the studies of Drezewieska et al. and Hoover et al. [33,38] who showed that disruption of rafts by cholesterol-depleting agents, such as β-cyclodextrin or nystatin, abrogated capping and signaling through the Fc-γ receptor and immunoglobulin receptors, respectively.

Junge et al. [45] extended these concepts by proposing that neutral sphingomyelinase-released ceramide was essential for capping of L-selectin in lymphocytes. Evidence for a mechanism by which rafts regulate receptor capping via the generation of ceramide was provided recently by the studies of Cremesti et al. [29] and Grassme et al. [46,47]. These investigations, which use the Fas and CD-40 receptors as a model, are discussed in more detail in Section 6.

In summary, these different studies suggest that rafts play a critical role in capping and activation of distinct receptors resulting in regulated signaling of diverse biologic outcomes, including apoptosis, mitogenesis, and immune signaling.

6. Ceramide, sphingomyelinase and rafts

Several lines of evidence suggest that membrane rafts are the specific sites for ceramide generation in response to various agonists and stress signals [28,29,48–50]. Liu and Anderson were the first to show that ceramide levels were elevated and sphingomyelin levels decreased in the caveolae compartment of human fibroblasts in response to interleukin (IL)-1β treatment [49]. Furthermore, this increase in ceramide was due to the activity of a zinc-independent acid sphingomyelinase, which was found to be enriched in those fractions. These investigators observed that the early increase in ceramide in response to IL-1β treatment correlated with decreased platelet-derived growth factor-induced thymidine uptake at 24 h. Similarly, Bilderback et al. [51] showed that sphingomyelin is hydrolyzed from the caveolae-enriched fractions only of NIH

3T3 fibroblasts in response to nerve growth factor (NGF). These authors also showed that the low affinity neurotrophin receptor $p75^{NTR}$ clustered in caveolae of fibroblasts over-expressing $p75^{NTR}$ and in caveolae of PC-12 pheochromocytoma cells. Most importantly, disruption of rafts with cholesterol-depleting agents abolished $p75^{NTR}$-dependent sphingomyelin hydrolysis and ceramide generation [52], defining the importance of intact caveolae for sphingomyelinase action during NGF signaling. Rafts have also been shown to be the sites of ceramide generation in response to heat shock and ionizing radiation. As stated above, Zundel et al. [28] showed that radiation-induced ceramide generation within caveolae, via acid sphingomyelinase (ASM), results in PI3K inhibition through the modulation of caveolin-1 function.

The role of different sphingomyelinases in the generation of ceramide within rafts is the subject of ongoing research. Activation of ASM vs. neutral sphingomyelinase (NSM) occurs differently in response to different stimuli [1]. ASM and NSM have both been detected in rafts [28,53]. Cremesti et al. have shown that ASM has the capacity to relocate rapidly from an intracellular compartment to the plasma membrane upon Fas stimulation [29]. Levade and his group showed that a substantial pool of NSM, about 22% of the total, resides in caveolae of human skin fibroblasts. NSM was also detected in rafts of fibroblasts from Niemann–Pick disease patients that lack ASM [53]. Further, after TNF stimulation, a portion of the TNF receptor moved into caveolae, while NSM exited, followed by a specific increase in ceramide and a decrease in sphingomyelin in that compartment [53], suggesting that ASM might be mediating sphingomyelin hydrolysis. The generation of ceramide in this compartment specifically in response to select agonists reinforces the idea that these microdomains may be crucial for the signaling of downstream effectors of ceramide action.

Recently, the role of membrane rafts in the signaling of the Fas and CD-40 receptors has been elucidated [29,46,47,54]. Our laboratory and that of Gulbins and co-workers showed that ceramide generation and intact rafts were essential for optimal Fas signaling and induction of apoptosis in B and T lymphocytes. Treatment of Jurkat T cells with cholesterol-depleting agents prevented ceramide generation and apoptosis induced by anti-Fas antibody for at least 9 h. Consistent with a requirement for ceramide generation for Fas signaling, Grassme et al. [54] showed that neutralization of surface ceramide with anti-ceramide antibody prevented Fas capping and apoptosis. Cells derived from Niemann–Pick disease patients were also defective in capping and apoptosis in response to Fas. The apoptotic response could be restored by the addition of nanomolar concentrations of exogenous C-16 ceramide. Thus ceramide was capable of reversing the phenotype without affecting the genotype, restoring receptor-mediated cell death.

Consistent with a role for membrane rafts in ceramide generation at the cell surface, ceramide could be detected by confocal microscopy as well as flow cytometry analysis of non-permeabilized cells using a new polyclonal anti-ceramide antibody, seconds after Fas stimulation. Confocal microscopy revealed that ceramide first appears localized in patches on the surface of cells after Fas stimulation. These patches rapidly merged to form larger platforms which contained the Fas receptor, ASM, and membrane raft constituents [54]. As the addition of long chain ceramides to the surface of Jurkat cells or generation of endogenous ceramide directly resulted in patch formation and coalescence into larger platforms, it appears that the driving force for capping may be ceramide itself.

Furthermore, this model of transmembrane signaling is not restricted to Fas, as it has been shown that ASM is essential

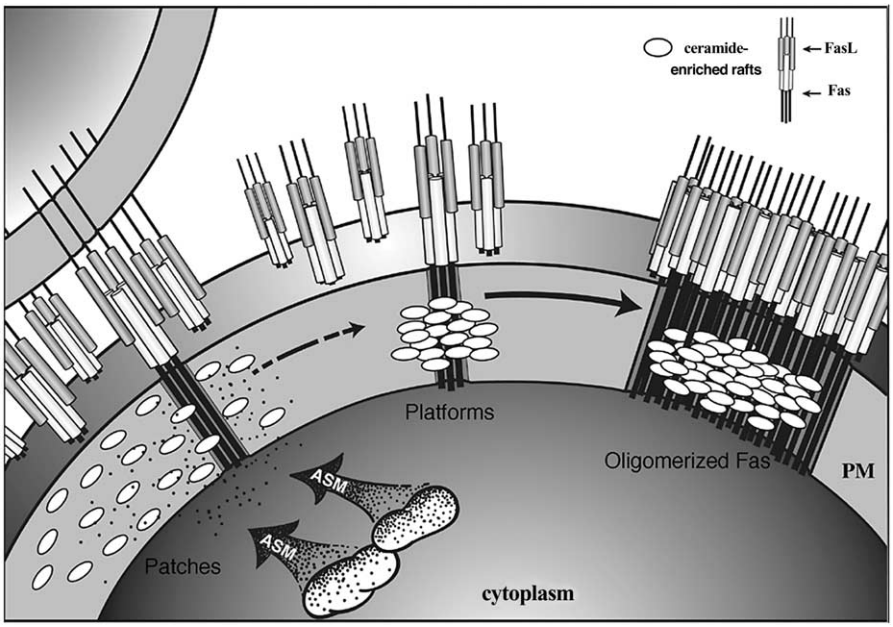

Fig. 1. Schematic representation of the events that precede capping of the Fas receptor. Seconds after Fas engagement, ASM translocates to the membrane and hydrolyzes sphingomyelin to ceramide within pre-formed rafts. Ceramide provides the driving force for the coalescence of rafts, which are sub-microscopic, into patches, and then into larger platforms. Only ligated Fas receptors are capable of entering and multimerizing within these platforms, resulting in oligomerization of the downstream effectors FADD/MORT-1 and pro-caspase-8 (not shown), an event required for transmission of the Fas death signal.

for the clustering of another TNF superfamily member, CD-40 [47]. Stimulation of lymphocytes via CD-40 ligation results in ASM translocation from intracellular stores to the membrane, followed by release of extracellularly-oriented ceramide, which in turn mediates CD-40 clustering in sphingolipid-rich membrane domains. Deficiency of ASM, destruction of sphingolipid-rich rafts, or neutralization of surface ceramide as with Fas prevents CD-40 clustering and CD-40-initiated cell signaling, indicating a strict requirement for clustering in rafts for propagation of the CD-40 signal [47]. Furthermore, the p55 TNF receptor, which is known to activate sphingomyelinase, has also been localized to rafts, whose disruption prevented TNF-induced cell death in Jurkat cells [55]. These studies provide solid evidence that rafts are the specific sites of ceramide generation, which appears essential for capping and signaling for some TNF receptor superfamily members.

These studies provide the basis for a proposed mechanism for the involvement of ceramide in the capping process as shown in Fig. 1 for Fas. Seconds after Fas engagement by ligand or agonistic antibody, ASM translocates from an intracellular pool to the outer leaflet of the plasma membrane to hydrolyze sphingomyelin and release ceramide. This ceramide results in the reorganization of rafts into larger platforms or macrodomains within which Fas clusters. Fas clustering presumably facilitates oligomerization of FADD and caspase-8, downstream Fas effectors which have to oligomerize to transmit the death signal. In the case of CD-40, the downstream effectors activated by the clustered receptor are those mediating CD-40 action.

These investigations begin to address the question of how ceramide promotes transmembrane signaling. We propose that the unique biophysical properties of ceramide discussed in the beginning of this review regulate the process of macrodomain formation from pre-formed microdomains. The capacity of ceramide to self-associate and its fusagenic properties may mediate the raft reorganization into macrodomains that is required for receptor capping, and perhaps for other signaling events. The reorganized rafts likely retain or restrict proteins differentially. In this regard, a subset of cellular proteins, such as KSR, Raf, phospholipase A_2, cathepsin D, protein phosphatase 2A, and PKC isoforms [2] are ceramide-activated, some through a ceramide-binding motif, the C1B domain [56]. Whether the C1B domain, or other as yet undefined ceramide binding sites, targets ceramide-activated proteins to regions of ceramide generation, such as rafts, is presently unknown.

7. Conclusions

Sphingomyelin–cholesterol microdomains or rafts form a stable lipid matrix, which acts as an ordered support for receptor-mediated signaling events. Sphingolipids play essential roles in the formation of these rafts through interaction with cholesterol, and in raft reorganization via ceramide. We suggest that the release of ceramide from sphingomyelin alters the dynamics of these rafts, and promotes macrodomain formation. These local changes in membrane microdomains would then drive signal transduction processes by allowing oligomerization of specific cell surface proteins, such as ligated receptors. As protein oligomerization is an almost universal requirement for transmembrane signal transmission, we propose that the reorganization of microdomains into macrodomains constitutes a new paradigm for transmitting signals across the plasma membrane.

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