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Cell Membranes: The Lipid Perspective

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Although cell membranes are packed with proteins mingling with lipids, remarkably little is known about how proteins interact with lipids to carry out their function. Novel analytical tools are revealing the astounding diversity of lipids in membranes. The issue is now to understand the cellular functions of this complexity. In this Perspective, we focus on the interface of integral transmembrane proteins and membrane lipids in eukaryotic cells. Clarifying how proteins and lipids interact with each other will be important for unraveling membrane protein structure and function. Progress toward this goal will be promoted by increasing overlap between different fields that have so far operated without much crosstalk.

Introduction

A basic problem in the study of biological membranes is the complex environment in which the constituent proteins and lipids are designed to function: an apolar membrane core separated from two distinct aqueous compartments by a complex aqueous-hydrophobic interface. Because of this unusual environment, integral membrane proteins extracted from cell membranes are usually not water soluble and therefore require complex isolation protocols to be studied by biochemical and biophysical means. In contrast, organic solvents can readily solubilize lipids, and this ease of handling made lipids the favorites in the early phase of membrane research. However, the introduction of DNA technology to identify the mRNAs encoding membrane proteins and the solving of the first structures of integral transmembrane (TM) proteins (Deisenhofer et al., 1984; Henderson and Unwin, 1975) swung the pendulum of membrane research to the proteins' side.

Now novel methods are revealing the remarkable diversity of the lipids in eukaryotic cell membranes and the study of protein-lipid interactions is picking up speed. The aim of this Perspective article is to give a lipid perspective on eukaryotic membrane research. In order to come to grips with how TM proteins interact with this lipid diversity, our message is that the lipid environments where proteins carry out their functions will have to be included in the studies of the biochemistry and structure biology of membrane proteins.

Membrane Protein and Lipid Diversity

As of October 2011, among ~71,000 protein structures available in the RSCB Protein Data Bank, only 1095 structures out of 285 unique proteins are those of membrane proteins (Membrane Protein Databank, Raman et al., 2006), although 20%–30% of all genes in genomes encode for TM proteins (Krogh et al., 2001). This is despite common efforts to develop new expression systems and crystallization strategies aiming to overcome bottlenecks hampering membrane protein research (Bill et al., 2011). Especially difficult has been the issue of how these proteins interact with lipids. The first shell of lipid around an integral protein is referred to as annular lipids (Lee, 2011), which rapidly exchange between the protein interface and the bulk of the membrane (Esmann and Marsh, 2006). However, some lipids are tightly bound and have been identified in structural studies (Hunte and Richers, 2008; Raunser and Walz, 2009). Another recent example is the crystal structure of a voltage-gated sodium channel (Payandeh et al., 2011). Nevertheless, these are only fascinating glimpses into the wide realm of protein-lipid interactions and how these are employed to modulate membrane protein function.

Most routinely used procedures for purification of membrane proteins for structural studies include steps to remove native lipids and these are not systematically added back prior to structural characterization thus taking native lipids out of the picture. This is mostly due to the challenges that native lipids pose to producing uniform and well-behaved protein samples, which are amiable to producing high-quality crystals. Nonetheless, as analytical techniques for the structural characterization of lipids improve, we are beginning to comprehend the astounding diversity of lipids that are present in biological membranes (Shevchenko and Simons, 2010; Wenk, 2010). Cells contain hundreds of different lipid species that can be categorized into three main classes: glycerophospholipids, sphingolipids, and sterols. Furthermore, additional complexity of eukaryotic lipids is generated by the many possible modifications of the hydrophilic head groups and the hydrophobic hydrocarbon tails (Figure 1).

The head group of a glycerophospholipid can be modified by the addition of various chemical moieties onto the sn-3 position of the glycerol backbone, leading to a number of different phosphatidyl lipids, such as phosphatidylcholine (PC), -ethanolamine (PE), -serine (PS), -glycerol (PG), -inositol (PI), or the unmodified phosphatidic acid (PA). The fatty acid chains in sn-1 and sn-2 positions can be variable in terms of length and numbers of double bonds (degree of saturation), and the linkage to the glycerol backbone can also be varied by ester, alkyl ether, or alkenyl ether bonds (Figure 1A).

Sphingolipids, unlike glycerophospholipids, are based on a lipid backbone, specifically sphingosine, which is amide-bonded to a fatty acid to form ceramide. The ceramide can be further modified and extended for instance with phosphocholine or glycans to form sphingomyelin and glycosphingolipids, respectively. Although combinatorial permutations of these lipid building blocks could theoretically generate tens of thousands of lipid species (Shevchenko and Simons, 2010; Yetukuri et al., 2008), naturally occurring membranes typically use "only" up to 1000 different species. A considerable part of our genome is

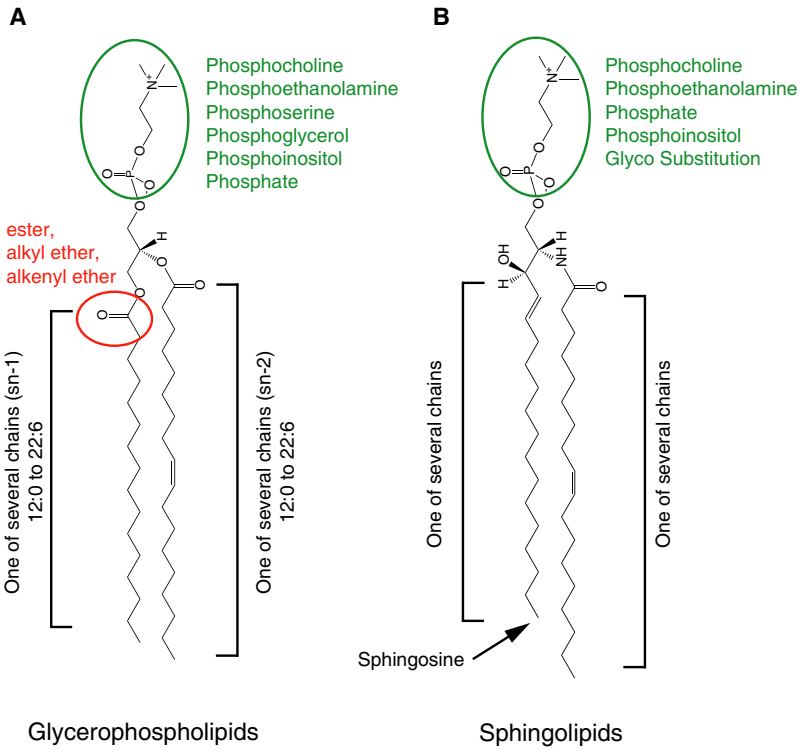

Figure 1. Chemical Diversity of Glycerophospholipids and Sphingolipids

Chemical modification of head groups (green circle), fatty acid chain length, and numbers of double bonds (degree of saturation) contribute to the diversity and complexity of (A) glycerophospholipid and (B) sphingolipids. The linkage in glycerol in the sn-1 in glycerophospolipids (red circle) increases the variety further. Instead of a glycerol, sphingolipids contain a backbone of sphingoid base, including sphingosine, which is amide bound to a fatty acid to form ceramide. For further insights about sphingolipid complexity, see Merrill (2011).

required to synthesize, metabolize, and regulate such a complex array of lipids, but we are far from understanding why this diversity is needed.

Making It to the Plasma Membrane

To understand protein-lipid interactions, it will be important to characterize the lipid environment into which membrane proteins are inserted, as well as the diversity of lipid environments, which proteins encounter during the intracellular journey to their final destination.

Virtually all eukaryotic membrane proteins start their lives at the endoplasmic reticulum (ER), where they become integrated into the ER membrane by the translocation machinery of the translocon. The translocation of a hydrophobic transmembrane domain (TMD) involves a stop signal of positively charged amino acids (positive-inside rule) that interacts with negatively charged glycerophospholipids in the cytoplasmic leaflet (von Heijne, 1989). In prokaryotes, lipid-protein interactions have been shown to play an important role in the generation of the correct protein topology with respect to the membrane (Dowhan and Bogdanov, 2009).

The translocation machinery is similar in eukaryotic and prokaryotic cells. One difference is that the eukaryotic TM-proteins have a variety of potential destinations from the ER membrane into which there are translocated, including the Golgi complex, endosomes/lysosomes and the plasma membrane (PM). The ER is capable of integrating all these proteins into its membrane despite the fact that they are often designed to function in membranes with different properties. For instance, the membrane spanning domains of proteins should match the thickness of the bilayer. However, the TMDs of PM proteins are longer than those of the ER and Golgi complex (Bretscher and Munro, 1993) and the ER lipid bilayer must adapt to the different TMDs to avoid hydrophobic mismatch. Eukaryotic cells have added sterols to their lipid repertoire and when proteins exit the ER, they encounter membranes with varying cholesterol content, increasing along the biosynthetic route over the Golgi complex toward the PM (van Meer et al., 2008). Cholesterol serves to both thicken and rigidify those membranes, accentuating potential hydrophobic mismatching between the hydrophobic protein TMDs and the lipid bilayer core. Theoretical studies have shown that this effect of cholesterol potentiates the intrinsic sorting capability of mismatched systems (Lundbaek et al., 2003). This prediction has been confirmed in recent experiments showing that shorter Golgi model TMDs segregate from longer PM-model TMDs when the cholesterol concentration is increased in model bilayers (Kaiser et al., 2011). Thus, the cholesterol gradient from the ER to the cell surface can regulate sorting of membrane proteins to their correct membrane site, while allowing broad-spectrum incorporation into the ER. In the ER, the bilayer adapts locally to newly synthesized proteins having different TMD lengths because the ER membrane is cholesterol poor and therefore more adaptable. Consistent with this idea, general protein translocation has been show to be inhibited by elevated levels of cholesterol in the ER membrane (Nilsson et al., 2001). In the Golgi complex, cholesterol concentration increases toward the trans-side, promoting sorting of shorter Golgi proteins from PM proteins with longer TMDs (Sharpe et al., 2010). However, hydrophobic mismatch might still play a role in regulating ER protein function. The optimal activity of Ca^{2+}-ATPase is observed in liposomes containing lipids with C18 fatty acids, shorter or longer chain lengths decrease activity (Lee, 2011). The functional state of a particular protein could potentially be adapted to an optimal bilayer thickness, allowing for passive silencing of protein function in transit to their final destination.

Another addition to the eukaryotic lipid repertoire can be found in the Golgi complex where ceramide-based sphingolipids are synthesized, including sphingomyelin and glycosphingolipids (Figure 1B). Sphingolipids introduce another sorting principle for proteins destined to the PM, based on preferential association of sphingolipids with cholesterol (Simons and Ikonen, 1997). Sphingolipid-cholesterol assemblies associate with specific PM

proteins to form dynamic nanoscale rafts, having the capacity to coalesce to larger platforms by crosslinking (Lingwood and Simons, 2010). This concept of dynamic and specific subcompartmentalization of the membrane has been revitalized by the development of high temporal and spatial resolution techniques to correlate the location of different molecular constituents in the plasma membrane. These methods are capable of observing glimpses of the elusive nanoscale state and following its functionalization into more stable assemblies involved in membrane trafficking, signal transduction, and many other membrane processes (Eggeling et al., 2009; Kusumi et al., 2011).

The pathways emanating from the trans-Golgi network use several different mechanisms to sort proteins for delivery to post-Golgi destinations. There is now strong evidence in yeast that one such pathway to the PM employs sphingolipids and sterols to assemble proteins into transport carriers. The most convincing result is that these carriers can be isolated in high purity and they are heavily enriched in sphingolipids and sterols (Klemm et al., 2009). As a result of these sorting processes, PMs are enriched in cholesterol, and sphingolipids.

Fine-Tuning Biological Function via Lipid Composition

These membrane-sorting processes occur in many eukaryotic cell types to generate and maintain the composition of lipids and proteins necessary to carry out specific organellar functions in the biosynthetic and endocytic pathways. However, there are even more dramatic instances of cellular membranes with specific lipid composition. One such example is the disk membrane of retinal rod photoreceptor cells. The major protein in disks is rhodopsin, the visual photo pigment and member of G protein coupled receptor (GPCR) family (Jastrzebska et al., 2011). These membrane disks are formed from the PM in the outer segment of the photoreceptor epithelial cells and have a unique fatty acid composition of phospholipids, with a remarkable 80 mol% of the omega-3 docosahexaenoic acid (22:6) (DHA) and also containing very long-chain fatty acids (up to C32-36). The function of rhodopsin has been shown to be dependent on these polyunsaturated fatty acids (Niu et al., 2004). Structural and spectroscopic studies have examined these protein-lipid interactions in detail with the conclusion that rhodopsin is constructed to accommodate DHA-lipids in grooves of its structure (Mihailescu et al., 2011).

How the disk membranes are generated from the PM of the rod cells remains a puzzle. Perhaps the budding of the disc membrane is regulated by a phase separation process in which sphingolipid-cholesterol rafts are excluded from the disc membrane, the protein composition of which is dominated by a single protein (rhodopsin) that interacts strongly with DHA-containing lipids. Consistent with this hypothesis of raft exclusion, the sphingolipid content of disks is less than 1%–2%, while cholesterol is lowered to 8%–10% of total lipids (Jastrzebska et al., 2011), in contrast to PMs of other cells, which generally contain between 30%–50% cholesterol.

An example of a cell membrane that has evolved in the opposite direction is the ocular lens membrane (Borchman and Yappert, 2010). The lipids of this membrane are characterized by an unusually high content of saturated fatty acids (Epand, 2003). The major lipid specie is dihydrosphingomyelin, which makes up almost half of the phospholipids. These lipids seem to be bound to lens proteins such that the overall organization is highly rigid. The lens is made up of membrane fibers formed from the PM of the lens epithelium. Using a similar principle as proposed for the formation of retinal rod disk membranes, the biogenesis of the lens fibers could be driven by a phase separation process, during which the raft phase, containing dihydrosphingomyelin, cholesterol, and lens proteins, segregates into the lens membranes.

Fine-Tuned Biological Function via Protein-Lipid Interactions

The unique lipid composition of a specific cell membrane could affect the function of a protein in two independent and distinct ways—either by tuning the bulk properties of the membrane to ensure the set of physical parameters required for proper protein function or by specific protein-lipid interactions, which could stoichiometrically and allosterically modify protein structure and function.

In mitochondria, one specific lipid constituent is cardiolipin and several mitochondrial proteins have been shown to be dependent on this lipid for activity (Arias-Cartin et al., 2011; Pfeiffer et al., 2003). In the PM, TM proteins could potentially interact with sphingomyelin and cholesterol. Examples of cholesterol-interacting proteins are GPCRs, the best studied of which is the β-adrenergic receptor (Cherezov et al., 2007), and caveolin, though its structure is not yet known. Another recent example is the elucidation of the structural basis of the activation of the inward rectifier K^+ channel. Here, phosphatidylinositol 4,5-bisphosphate, a plasma membrane lipid, which was added to the purified protein prior to crystallization, was found to modulate protein conformation (Hansen et al., 2011). Our hypothesis is that some TM proteins will interact specifically with lipids that are characteristic for the membrane where they carry out their function. From this, one would predict that there are PM proteins that would specifically interact with sphingolipids. However, there has so far been no protein structure in which sphingolipids can be observed.

In addition to sphingomyelins, which contain fatty acyl chains of different length, saturation, and hydroxylation, mammalian cells synthesize hundreds of sphingolipid species, carrying different carbohydrate head groups. Little is known of the function of this diversity. An interesting feature of glycosphingolipids is that the conformation of the glycan head group can be modulated by the lipid environment. It was recently demonstrated that the presence of cholesterol induces a tilt in the glycolipid head group of the ganglioside GM1, a neuraminic acid-containing glycosphingolipid, resulting in a reduction of cholera toxin binding (Lingwood et al., 2011). The property of head group tilting and changes in ganglioside identity and recognition turned out to organize erythrocyte blood group presentation and glycolipid receptor function during the activation of sperm fertility, suggesting that lipid "allostery" is a means to regulate membrane recognition processes and protein interaction (Lingwood et al., 2011; Yahi et al., 2010). Another interesting example of lipid allostery is the regulation of tyrosine kinase receptor activity by gangliosides. Although gangliosides have been reported to affect growth factor receptor function (Miljan and Bremer, 2002), this has received little attention in recent literature on growth factor

receptor signaling (Lemmon and Schlessinger, 2010). We took the epidermal growth factor (EGF) receptor as a showcase to find out directly whether gangliosides modulate receptor activity as had been claimed. The receptor was reconstituted into liposomes of different lipid composition. The lipid composition had no effect on the equilibrium ligand-binding properties of the EGFR (Coskun et al., 2011). However, a ganglioside dramatically inhibited kinase domain activation. The effect was very specific and was seen only with the ganglioside GM3, which completely abolished autophosphorylation of the receptor. The inhibitory effect could be demonstrated only in proteoliposomes tuned to phase separate into liquid-ordered (Lo) and -disordered (Ld) domains (Mouritsen, 2011) (Figure 2). These data suggest that GM3 can regulate the allosteric structural transition from an inactive to a signaling EGFR dimer and demonstrate the potential importance of glycosphingolipid-protein interactions, mostly neglected in the cell and structural biology field so far.

Outlook

It is our contention that the protein and lipid camps have to join efforts to unravel how cell membranes work. We only can begin to understand how membrane proteins perform their function if they are studied within the context of the lipids that surround and often regulate them. Therefore, a prerequisite is to find out which lipids are present in the membrane of interest. This is not an easy task since the art of organelle purification has still not come of age. Nevertheless, analytical methods to quantitatively characterize comprehensive lipidomes are available (Shevchenko and Simons, 2010). Importantly, these new methods require only minute amounts of material for analysis, compared with previous lipid analysis methodology that had excessive demands in that respect. Also, solubilization methods of membrane proteins have to be refined not to extract all lipids surrounding the protein and such isolation attempts can now be easily followed by the sensitive mass spectrometric methods available not only for protein, but also for lipid identification and quantification. Novel methods have been introduced using "click chemistry" to identify lipids interacting with proteins (Haberkant and van Meer, 2009). These methods employ new types of photoactivatable lipid probes, containing "clickable" reporter molecules, which can be enriched for mass spectrometric identification after crosslinking to membrane proteins.

Further characterization by biochemical, biophysical, and structural methods will require a library of natural lipids covering the most important classes and species. Although many of these are already commercially available, others have to be purified or synthesized. Reconstituting membrane protein function by recapitulating the native lipid environments of the proteins should become a routine tool in membrane research. It is to be expected that specific lipids will have the potential to change the conformation of proteins, but it will also be essential to take membrane thickness and other physical properties of the lipid surroundings into account.

Besides advances in electron crystallography (Wisedchaisri et al., 2010), new tools in membrane protein structural studies include bicelles, lipidic cubic phases, and nanodiscs (Bayburt and Sligar, 2010; Caffrey, 2009; Faham and Bowie, 2002), and these could be refined by including strategic lipids from native membranes. A recent big success that presents such an example for eukaryotic proteins was the unraveling the β_2 adrenergic receptor as a dimer, in which the dimer interface is composed mainly of lipids, with two cholesterol molecules and two palmitic acid molecules forming the majority of the interactions (Cherezov et al., 2007). This protein, like many other GPCRs, is palmitoylated during transport to the cell surface. Crystallization was achieved by the use of lipidic cubic phases (Landau and Rosenbusch, 1996) to which cholesterol had been added. Subsequently using the same method, several other GPCR structures were solved (Cherezov, 2011). Could one argue that including those lipids that the GPCRs encounter during normal intracellular transport into the crystallization protocol facilitated these successes?

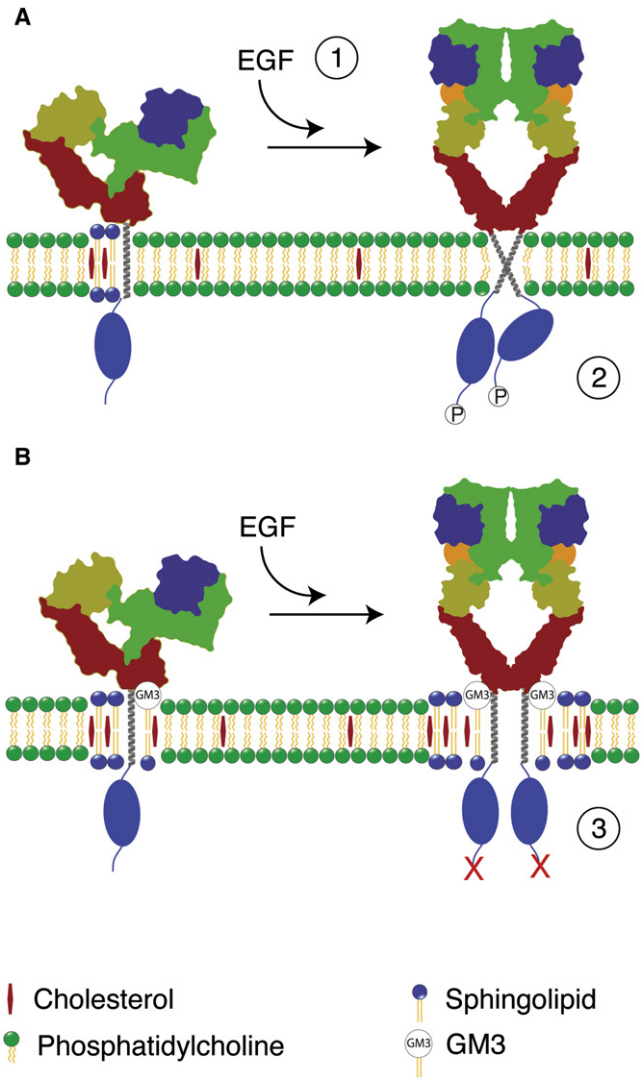

Figure 2. Proposed Allosteric Inhibition Scheme of EGF Receptor Kinase Activation by the Ganglioside GM3

(A) EGF receptor association with sphingolipid/cholesterol domains prevents aberrant activation of EGFR signaling. EGF ligand binding promotes EGFR dimerization (1) leading to the formation of an active dimer (2) (Jura et al., 2009).

(B) When GM3 is present in the bilayer, the direct association of GM3 with the EGFR ectodomain leads to the inactivation of the EGFR kinase activity (3).

The fascination of membrane research is that the functionality of the cell membrane is dependent on the carefully orchestrated and mutually interdependent properties of lipids and proteins. The proteins and lipids in membranes form collectives, the biology and physics of which will remain a challenge and a source for new insights in years to come.

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