Biochimica et Biophysica Acta xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Cell membranes: A subjective perspective☆

Kai Simons

Max-Planck-Institute of Molecular Cell Biology and Genetics, Pfotenhauerstr. 108, 01307 Dresden, Germany

ARTICLE INFO

Article history:
Received 26 November 2015
Received in revised form 19 January 2016
Accepted 21 January 2016
Available online xxxx

Keywords:
Lipids
Membranes
Lipid rafts
Lipid–protein interactions
Electrostatic properties
Mass spectrometry

ABSTRACT

Cell membranes have developed a tremendous complexity of lipids and proteins geared to perform the functions cells require. The lipids have for long remained in the background and are now regaining their role as important building blocks of cells. Their main function is to form the matrix of our cell membranes where they support a variety of functions essential for life. This 2-dimensional fluid matrix has evolved unexpected material properties that involve both lipid–lipid and lipid–protein interactions. This perspective is a short summary of the challenges that this field faces and discusses potential ways and means for coming to grips with the properties of this incredible fluid. This article is part of a Special Issue entitled: Biosimulations. Guest Editors: Ilpo Vattulainen and Dr. Tomasz Róg

An astounding feature of cell membranes is that although the basic bilayer can be self-assembled from only one single glycerophospholipid species, the membranes in our cells are made of hundreds of different lipids. Today we are able to analyze this complexity with a new generation of mass spectrometers that cope with lipid diversity with unprecedented precision and efficiency. The challenge is now to understand why our cells need to have so many lipids in their membranes. How do the lipids mingle and interact with the proteins in the membranes? Why does the lipid matrix differ in the various membranes of the cell? How do lipids contribute to membrane architecture and cell morphogenesis? How do membranes shape themselves into protrusions, invaginations, tubules and vesicles or form cubic membranes? Or how do membranes sub-compartmentalize to form dynamic subdomains? New methods are being generated such as powerful novel imaging methods based on a plethora of fluorescent probes but we are far from understanding how cell membranes can perform all these feats. This perspective will summarize what we can do to meet this challenge.

1. Lipids in the cell membranes: composition and location

It is now well established that cell membranes are composed of both proteins and lipids. However, the lipids were long neglected because of lack of adequate methods to analyze them and probe their role in biochemistry, biophysics and cell biology. When the Gordon Conference for Molecular Membrane Biology was started over 30 years ago, the program was focused on the proteins; the lipids had a backstage role. This trend continued for years. But now with the introduction of new methodologies, lipids are becoming more accessible and their role in membranes is being increasingly explored. Most lipids have polar head groups and hydrophobic hydrocarbon chains that differ in length, hydroxylation and saturation. So far this chemical diversity has received little attention. Most studies focused on lipid classes (PC, PE, PA, PS, PI, SM etc.) and not on species and subspecies. We only have a vague understanding of which enzymes are responsible for synthesizing different lipid subspecies and regulating their metabolism. These are now tasks that can be tackled by our new lipidomics tools to analyze lipidomes quantitatively and with high coverage [1,2]. Not only traditional genetics but also CRISPR/Cas9 methodologies can be mobilized to systematically analyze lipid metabolism at this level in different cells and organisms [3]. We know roughly where different lipid classes are made [4]. The endoplasmic reticulum (ER) is the main synthesis site but many other organelles contribute to the generation of the lipid spectrum in cells. The newly synthesized lipids are distributed both by membrane traffic and by direct delivery across contact sites between organelles, thereby establishing and maintaining the lipid compositions that are characteristic to each cell type and their variety of membranes.

We are starting to appreciate that all lipid subspecies are probably as tightly regulated as sterols are. This implies that lipid metabolism has to be stringently controlled. How this is achieved remains poorly understood. Clues are starting to emerge about possible sensing mechanisms that can respond both to the chemical structure [5] and to the physical properties [6,7] of the lipids and of the bilayer. However, this is only a humble beginning. These studies will occupy the field for years to come. We are lacking methods to isolate organellar membranes in high enough purity so that their lipidomes can be measured. Progress in this area has been dismally slow. The tools to analyze membrane

☆ This article is part of a Special Issue entitled: Biosimulations. Guest Editors: Ilpo Vattulainen and Dr. Tomasz Róg

E-mail address: simons@mpi-cbg.de.

http://dx.doi.org/10.1016/j.bbamem.2016.01.023
0005-2736/

lipidomes are available but the methods for organelle purification have to be overhauled. Take the secretory pathway as an example. Newly synthesized proteins and lipids are transported from the ER to the cis-side of the Golgi complex, moved to the trans-side, and then sorted in the trans-Golgi network (TGN) into different transport vehicles for delivery to the plasma membrane (PM) and other destinations [8]. Each of these pathways transports different lipid and protein cargoes [9]. A gradient of cholesterol is established, starting low in the ER where the sterols are synthesized and increasing over the Golgi complex, reaching its peak in the PM. Sphingomyelin glycosphingolipids, and gangliosides are produced in the Golgi complex and transported to the PM where their concentration is the highest [8]. Also other lipids such as PS are enriched in the PM. What is lacking are quantitative lipidomes with subspecies coverage to show how the different lipids are distributed over the different organellar membranes. We now need to follow how the whole spectrum of newly synthesized lipid subspecies are distributed over each cellular membrane and how the cellular lipids add up to give each cell membrane its unique identity.

Progress towards characterizing temporal and spatial remodeling of the cellular lipidome has been slow, but emerging observations show promise to open new frontiers in understanding cellular processes. So far only two populations of transport vesicles have been isolated in high enough purity to allow lipid analysis. Brügger et al. [10] isolated COPI vesicles from the Golgi complex *in vitro* and demonstrated the enrichment of SM 18:0. Later it was shown that this SM lipid binds to the p24 protein of the COPI vesicles and regulates the activity of the transport machinery [11]. Klemm at al. [12] purified post-Golgi transport vesicles from yeast and demonstrated that the glycosphingolipid species were specifically enriched by sorting in the TGN [13]. The data gave direct evidence for the role of the Golgi complex in producing the gradient of glycosphingolipids, peaking in the PM.

2. Protein–lipid interactions

Understanding the lipid environment where membrane proteins carry out their function is a starting point for analyzing how lipids contribute to modulating protein function in membranes. Understanding protein–lipid interactions will be key to understanding membrane organization and function. Membrane research was long divided into two camps that rarely met. The protein camp was the dominating one and caught most of the excitement. It bears repeating that the lipid camp was isolated and mostly ignored. This dismal situation has changed in recent years. It has now finally dawned on the field that proteins and lipids need to be studied together. Most importantly, new methods have generated tools that have the capacity of analyzing how lipids interact with proteins. Sophisticated mass spectrometric methods can now unravel integral membrane proteins in complex with specific lipid ligands [14]. The binding of peripheral proteins to specific lipids can be measured by a number of novel screening methods. Chemically engineered lipids can be employed to identify specific protein binding partners [15].

Not only x-ray crystallography but also electron cryo-microscopy is entering the field of protein lipid interactions [16,17]. CryoEM is the only method that can deliver structures at atomic resolution revealing how proteins interact with lipids in the membrane itself. The data emerging show that lipids form a continuous shell around the transmembrane domains of the protein. They also mediate contacts between protein subunits. The lipid hydrocarbon chains localize to grooves on the protein transmembrane surface to form an electrochemical seal across the membrane; they occupy the holes that proteins make in the bilayer. Interestingly, electron crystallography can also provide a measure for how tightly bound the lipids are. The crystallographic B-factor represents the statistical disorder of the protein–lipid structure [17]. If lipids are loosely interacting, representing bulk lipids associating to protein, the B-factor is higher than for those lipids that are specifically bound to the protein. Another useful feature of electron crystallography is that the electrical charge of the protein can be precisely localized. These exciting developments are bound to stimulate this important area of membrane research.

3. The structure and dynamic organization of cell membranes

The most challenging feature that membrane research faces, is the dynamic organization of cell membranes. It was first in the 1970s that it became clear that the lipids and the proteins can move laterally in the lipid bilayer i.e. that cell membranes are fluids [18]. At that time, it was also demonstrated that the lipids are organized asymmetrically in the two leaflets of the bilayer [19]. The lipids are flipped by specific flippases from one leaflet to the other to maintain the correct asymmetry under considerable energy expenditure [20]. Another intriguing feature was revealed already in the 1960s by Vittorio Luzzati who demonstrated that lipid bilayers can fold into different polymorphisms with specific symmetries [21]. These are highly curved 3-dimensional structures that display periodic cubic structures. This remarkable property showed that lipids have a unique capability to form different architectures. Also important is that the transition from a planar bilayer structure into cubic membranes requires little energy and this feature provides ample opportunity for cells to dynamically organize themselves. The only polymorphism that has entered biology are the cubic membranes [22,23] and my prediction is that we will see more of Luzzati's work in years to come. Cell membrane architecture is bound to be an important feature in generating form and shape in cellular architecture.

The focus of membrane research has so far been on local modulations of membrane organization. For example, how do membranes form vesicles and tubules and how do these fuse with the correct target membrane? As in so many other areas of biological research, the main achievement so far has been to identify the proteins – the parts list – regulating these processes and to characterize these players functionally. The role of the lipids was so far more or less neglected. Obviously, electron crystallography is starting to provide exact information on the interplay between the proteins and the lipids involved. However, this research is still in its infancy.

An interesting emerging area is to define how physical parameters such as membrane charge density, membrane curvature and lipid packing and thickness are being employed in regulating membrane function [24]. Using different surface probes and protein markers as well as by analyzing lipid composition, two territories have been defined in the secretory pathway. One is the early secretory pathway (the ER, nuclear envelope and the cis-Golgi). This territory is enriched in lipids, having monounsaturated acyl chains. It is also low in cholesterol and sphingolipids. This lipid composition is disordered and leads to voids and defects in the bilayer, thus constituting a membrane environment that allows the accommodation of different polypeptide chains in the bilayer. These are important properties for translocation and folding in the ER and for transport to the next station in the pathway, the Golgi complex [24–26]. The late secretory pathway (TGN, PM and the endosomes) forms the second territory. This is characterized by high electrostatics and by lipids that form more tightly packed and ordered bilayers. Generally there is gradient of lipids that have more saturated hydrocarbon chains (acyls and sphingosines) in the secretory pathway, peaking towards the PM. Negatively charged PS, phosphoinositides, cholesterol and sphingolipids are enriched in this territory [8]. Interestingly, PS in the luminal leaflet in the ER and the Golgi complex and is flipped to the cytosolic leaflet in the TGN [27]. PS is maintained exposed cytosolically in the PM and in the endosomes. The negatively charged cytosolic membrane surface facilitates binding of many peripheral proteins, required for different signal transduction processes and for regulating membrane shape and traffic [24].

Another interesting feature is the role of polyunsaturated acyl chains such as C20:4 and C22:6 [28]. They are known to be enriched in lipids of the photoreceptor membranes and synaptic vesicles. These fatty acids

Please cite this article as: K. Simons, Cell membranes: A subjective perspective, Biochim. Biophys. Acta (2016), http://dx.doi.org/10.1016/j.bbamem.2016.01.023