Cell Calcium 64 (2017) 72–82

Contents lists available at ScienceDirect

Cell Calcium

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

Review

Quantifying lipid changes in various membrane compartments using lipid binding protein domains

Péter Várnai[b], Gergő Gulyás[b], Dániel J. Tóth[a,b], Mira Sohn[a], Nivedita Sengupta[a], Tamas Balla[a,*]

[a] Section on Molecular Signal Transduction, Program for Developmental Neuroscience, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, United States
[b] Department of Physiology, Faculty of Medicine, Semmelweis University, Budapest, Hungary

ARTICLE INFO

Article history:
Received 29 November 2016
Received in revised form
28 December 2016
Accepted 29 December 2016
Available online 31 December 2016

Keywords:
Phospholipid
Phosphoinositide
Membrane
PH domain
Phospholipase C
PX domain
FYVE domain
Fluorescent imaging
TIRF microscopy
BRET analysis
FRET analysis
Confocal microscopy

ABSTRACT

One of the largest challenges in cell biology is to map the lipid composition of the membranes of various organelles and define the exact location of processes that control the synthesis and distribution of lipids between cellular compartments. The critical role of phosphoinositides, low-abundant lipids with rapid metabolism and exceptional regulatory importance in the control of almost all aspects of cellular functions created the need for tools to visualize their localizations and dynamics at the single cell level. However, there is also an increasing need for methods to determine the cellular distribution of other lipids regulatory or structural, such as diacylglycerol, phosphatidic acid, or other phospholipids and cholesterol. This review will summarize recent advances in this research field focusing on the means by which changes can be described in more quantitative terms.

Published by Elsevier Ltd.

Contents

* Corresponding author at: Bldg 35, 35 Convent Drive Rm. 2D842, Bethesda, MD 20892, United States.
E-mail address: ballat@mail.nih.gov (T. Balla).

http://dx.doi.org/10.1016/j.ceca.2016.12.008
0143-4160/Published by Elsevier Ltd.

1. Introduction

The genomic era combined with GFP technology has yielded unprecedented progress in understanding the function, cellular distribution and interaction of protein signaling networks and their regulation both in normal cells and in disease states. Many proteins have been characterized at the structural level providing important clues about their functions or dysfunctions as defined by their atomic architecture. There has also been an enormous progress in analyzing the lipid composition of tissues and cells revealing a complexity that is hard to comprehend. Each major lipid class is represented with a repertoire of molecular species with various acyl side chain lengths and saturation and in addition to ester lipids, ether lipids and sphingolipids add to this enormous wealth of lipid variety. What is the significance of this range of variability when it comes to building the complex membrane architecture of eukaryotic cells? Where do enzymatic reactions that contribute to this heterogeneity take place and how the lipids move around in the cell's aqueous environment? How do cells decide whether to build membranes, store lipids or use them as energy source? These questions and many more, although not new, keep coming back to the frontiers as more and more signaling processes are recognized to require active participation of membranes not only as localization platforms but regulatory ones as well.

Most of our knowledge on lipid enzymology and cellular distribution has been based on classical membrane fractionation studies. However, now it is clear that several specific enzymatic reactions of lipid conversions are catalyzed by multiple enzymes encoded by distinct genes or by splice variants originating form a single gene. The cellular localization of the enzymes can be determined relatively easily with our modern tools, but as it turns out the lipid products of these enzymes can be rapidly transferred to other cellular compartments and this process often controls the metabolic flux through these pathways. Such transport-coupled enzymatic reactions, however, also mean that cell fractionations can lead to misleading conclusions concerning the distribution of lipids as it relates to an intact cell. We have to come to realize that we lack solid knowledge about the cellular distribution of even the most common phospholipids, such as phosphatidylcholine (PC), phosphatidylethanolamine (PE) or phosphatidylinositol (PI) in spite of the fact that we more or less know where the enzymes that make these lipids are found. We have even fewer clues about the distribution of the various side-chain variants of these phospholipids or of any other lipid classes and the compartments where fatty acid remodeling takes place.

Several approaches have been used to find answers to these questions over the last 40 or so years. Fluorescent derivatives of lipids have been developed and added to the cells to follow their localization movements and metabolic fate [1,2]. While these studies have made great contributions to our understanding how these lipids behave, there are some caveats regarding their reliability. The fluorescent tag, which is attached to the lipid headgroup or acyl side chain has a major impact on the behavior of the lipid altering its partitioning between various membranes or between the two leaflets or in the aqueous phase. Moreover, lipids added from the outside may not follow the same metabolic routes that are used by their endogenous counterparts. These caveats are being addressed by a new generation of chemical biological tools that address the means of delivery as well as the minimalization of changing the hydrophobic character of side chains [3–5].

An additional approach that has gained popularity is the use of antibodies raised against specific lipid species [6,7]. In addition to the usual problems with specificity, the disadvantage of this approach is that it requires fixation so live cells studies cannot be performed. Still, anti phospholipid antibodies have also been providing us with very useful information. They have a great advantage over some other methods in that they show the distribution of the endogenous lipids without distortions caused by adding exogenous lipids or expressing lipid-binding domains (see below). One area of caution is the largely unknown effects of the various fixation procedures on the physicochemical states or distribution of the lipids. More and more data suggest that lipids remains mobile even when cells are fixed with conventional methods used for protein detection. Moreover, multivalent detection by antibodies can cluster the still mobile lipids creating false conclusions regarding clustering or other localization features [8–11]. Nevertheless, this approach will remain an important one especially when combined with the use of the lipid binding modules (see below) in fixed cells as "antibodies". These latter ones have been extended for use at the EM level [12–14].

The third approach to determine where lipids are in the cell and how they change upon cellular responses was born out of the realization that many proteins contain domains for lipid recognition that can determine their cellular localization. Such lipid recognizing domains include pleckstrin homology (PH) domains, C1 or C2 domains (see below). In some cases these isolated domains fused to fluorescent proteins can specifically recognize lipid species in the membrane allowing the visualization of these lipids in live cells. The great advantage of these tools is that they can be used in intact cells allowing kinetic analysis of changes in real time. A disadvantage of this approach is that it requires the introduction of a protein that will bind to the lipids and disturb the physiology of the cells and distort the amount and distribution of the lipid in question. In addition, there is often an additional interaction (mostly with proteins) that contributes to the membrane attachment of these protein modules that complicates the interpretation of the data obtained. These caveats have received ample coverage in many reviews written by us [15–17] and others [18–21]. In spite of these limitations, this method, when used with caution, has contributed significantly to our understanding of the distribution and functions of many lipid species.

In addition to fluorescence-based microscopy, there are additional labeling-free methods that do not require introduction of any probes to get information about lipid distribution in cells or tissues. Differential interference contrast (DIC) microscopy and phase contrast microscopy has been widely used to resolve structural details of cells or tissues. These techniques exploits differences between the refractive indices of various lipid membranes but provide no information on lipid composition. To distinguish chemically specific structures one needs to generate chemical contrast, which can be achieved by analysis of vibrational spectra of molecules. Coherent anti-Stokes Raman scattering (CARS) microscopy exploits this principle and has been particularly used for analysis of lipid distribution in cells or tissue. It is beyond the scope of this review to go to more details regarding this technique. More on these label-free methods can be found in [22].

CARS microscopy requires special instrumentation and understanding the complex analysis process, which has limited its wider appeal. Therefore, fluorescence-based microscopy methods are still more popular. Moreover, the increasing repertoire of ever improv-