Biochimica et Biophysica Acta 1841 (2014) 1022–1030

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

Fat & fabulous: Bifunctional lipids in the spotlight ☆

Per Haberkant [a,*], Joost C.M. Holthuis [b,1]

[a] European Molecular Biology Laboratory, Meyerhofstrasse 1, 69117 Heidelberg, Germany
[b] Molecular Cell Biology, University of Osnabrück, Barbarastrasse 13, 49076 Osnabrück, Germany

ARTICLE INFO

Article history:
Received 2 December 2013
Received in revised form 2 January 2014
Accepted 5 January 2014
Available online 15 January 2014

Keywords:
Photoaffinity labeling
Protein–lipid interaction
Proteomics
Imaging
Click chemistry

ABSTRACT

Understanding biological processes at the mechanistic level requires a systematic charting of the physical and functional links between all cellular components. While protein–protein and protein–nucleic acid networks have been subject to many global surveys, other critical cellular components such as membrane lipids have rarely been studied in large-scale interaction screens. Here, we review the development of photoactivatable and clickable lipid analogues–so-called bifunctional lipids–as novel chemical tools that enable a global profiling of lipid–protein interactions in biological membranes. Recent studies indicate that bifunctional lipids hold great promise in systematic efforts to dissect the elaborate crosstalk between proteins and lipids in live cells and organisms. This article is part of a Special Issue entitled Tools to study lipid functions.

1. Introduction

Lipids are among the most abundant cellular metabolites with an overwhelming diversity in structure and function. The lipidome of a cell typically comprises over 1000 lipid species [1]. Most of these belong to one of three major classes: glycerolipids, sphingolipids and sterols. A challenging problem is to understand how this enormous diversity is exploited at the biological level. Besides their fundamental role in membrane assembly and architecture, lipids can provide anhydrous stores of energy and serve as signalling molecules in a multitude of cellular processes. Lipids are non-randomly distributed among the various cellular organelles as well as within individual organelles. Their correct distribution relies, at least in part, on a tight spatial organization of lipid-metabolizing enzymes and lipid transporters [2], suggesting an elaborate network of lipid–protein interactions. The importance of these interactions is further underlined by the large variety of protein domains that evolved to bind particular lipids and the list of human disorders that have been linked to mutations in these domains [3]. Recent work suggests that some of these lipid-binding domains act as coincidence sensors for distinct lipid species [4]. Other studies revealed an unexpected high level of specificity by which some integral membrane proteins recognize and rely on individual lipid species [5,6]. These findings emphasize a need for systematic surveys to map the lipid–protein interaction network. Following a brief summary on how lipids and proteins may crosstalk in cellular bilayers, we will highlight some of the conventional methods that have been used to map lipid–protein interactions on a global scale. Next, we will focus on the emergence of photoactivatable and clickable lipids as promising chemical tools in large-scale efforts to unravel the lipid–protein interactome.

2. Lipid–protein cross-talk

Membrane proteins–i.e. proteins acting on or in cellular bilayers–perform a multitude of cellular functions, from signalling to transport. There are ample indications that the lipid molecules in the bilayer influence the activity of membrane proteins beyond their role as bulk solvent. This can occur in a variety of ways. Membrane proteins may respond to changes in the generic physical properties of the lipid bilayer such as membrane fluidity, hydrophobic thickness, surface charge and intrinsic curvature. For instance, the thermosensor DesK from *Bacillus subtilis* appears to sense the thickening of the lipid bilayer as the temperature drops. This is thought to force a cluster of hydrophilic residues into a more hydrophobic environment, resulting in a conformational change that triggers the sensor's autokinase activity to activate expression of a lipid desaturase, DesR [7]. Other proteins respond to lipid packing defects generated by high membrane curvature or the presence of conical lipids like diacylglycerol (DAG) and phosphatidylethanolamine (PE) in a flat bilayer. These proteins typically contain an amphipathic helix that acts as a lipid-packing sensor [8]. Such lipid-packing sensors play a critical role in the recruitment and activation of various peripheral membrane proteins, including GTPase-activating protein Arf1GAP1 [8] and CTP:phosphocholine cytidylyltransferase [9], the rate limiting enzyme in phosphatidylcholine (PC) biosynthesis. Other proteins are recruited onto the membrane surface through lipid–protein interactions mediated by specialized lipid-binding domains. Well-studied

☆ This article is part of a Special Issue entitled Tools to study lipid functions.

* Tel.: +49 6221 3878498.
E-mail addresses: per.haberkant@embl.de (P. Haberkant), joost.holthuis@biologie.uni-osnabrueck.de (J.C.M. Holthuis).
[1] Tel.: +49 5419 697140.

1388-1981/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbalip.2014.01.003

examples include the C1 and C2 domains present in DAG-responsive members of the protein kinase C (PKC) family and the pleckstrin homology (PH) domains found in a large variety of proteins that possess specificities for phosphoinositides (PIPs) phosphorylated at different sites within the inositol ring [10]. This type of protein–lipid interaction is common in cell signaling and membrane trafficking. In fact, our current understanding of lipid–protein cross-talk is largely dominated by specific interactions between peripheral membrane proteins and low abundant lipids such as DAG, phosphatidic acid (PA) and PIPs, whose local synthesis and interconversion exert tight spatiotemporal control over a wide array of cellular processes [11].

In contrast, only few examples of specific lipid–protein interactions mediated by lipid-binding domains buried in the membrane interior are known. X-ray crystallography revealed lipid molecules that are bound at specific sites between the alpha-helical transmembrane segments of various ion channels and multi-subunit complexes involved in bioenergetics [12,13]. These so-called structural lipids are regarded as co-factors rather than solvent. Of note is the requirement of cardiolipin, a lipid synthesized in the inner membrane of mitochondria, for supercomplex formation between respiratory chain complexes III and IV [14,15]. A particularly intriguing example concerns the allosteric regulation of p24, an abundant integral membrane protein of COPI vesicles, by a single molecular species of sphingomyelin, SM18:0 [5]. An assembly of four residues termed the "molecular species-determining" (MSD) motif was found to critically influence the specificity of interactions with SM18:0. A p24 MSD point mutant with compromised SM18:0 binding was defective in sustaining normal COPI-mediated membrane trafficking. Bioinformatics approaches identified MSD-like motifs in the membrane spans of various other integral membrane proteins, including the gamma-interferon receptor INGR1 [5]. Given the enormous diversity of lipid species and integral membrane proteins in cellular bilayers, specific lipid–protein interactions that occur in the membrane interior are unlikely to be rare events. The emerging notion that membrane function in cells relies on an elaborate cross-talk between lipids and proteins has raised considerable interest in methods to map lipid–protein interactions on a global scale.

3. Mapping lipid–protein interactions

Several proteome-wide methods have been developed to detect specific lipid–protein interactions (Fig. 1). For instance, Zhu et al. used a yeast proteome microarray comprising 5.800 affinity-purified yeast proteins immobilized on glass slides to search for PIP-binding proteins [16]. To this end, the proteome microarray was incubated with different PIP-containing liposomes that were spiked with biotinylated PE for detection. This approach led to the identification of 150 lipid-binding proteins, of which one-third showed a preference towards one or more PIPs in comparison with the carrier lipid PC. In an inverted setup, lipids can be covalently coupled to resins or other solid supports to allow identification of their protein binding partners by affinity purification [17]. In order to catalogue lipid–protein interactions in yeast, Gallego et al. used miniaturized lipid arrays to determine lipid-binding fingerprints of 172 tagged proteins, including 91 with predicted lipid-binding domains [4]. To this end, 56 different lipids and their metabolic intermediates were sprayed on nitrocellulose supports and analyzed for their ability to capture the tagged proteins. Besides the identification of hundreds of novel lipid–protein interactions, the screen also revealed that some PH domains can act as coincidence sensors of phosphoinositides and phosphorylated sphingolipids.

A major drawback of screens using lipids immobilized on solid supports is that such lipids are not presented in their native state. For example, interactions where the lipid has to enter a deep hydrophobic binding pocket within the protein are likely to be missed. To circumvent this, the Gavin group went on and established a liposomal microarray-based assay in which lipids were sprayed on a thin layer of agarose. Assembled in a microfluidic chamber, liposomes were formed upon hydration. Lysates of fluorescent-tagged lipid-binding proteins were subjected to nearly simultaneously monitor their preferential binding to 120 different liposomal membranes [18]. In a different setup, Maeda et al. purified protein–lipid complexes from extracts of yeast strains expressing physiological amounts of proteins fused to a tandem-affinity purification tag [19]. This procedure was applied to 13 yeast lipid transfer proteins, namely six Sec14 homologs and seven oxysterol-binding homology (Osh) proteins. Lipids bound to purified LTPs were extracted by organic solvents and identified by thin layer chromatography and mass spectrometry. Unexpectedly, two Osh proteins, Osh6 and Osh7, were found to bind phosphatidylserine (PS) and mediate non-vesicular PS transport from the endoplasmic reticulum (ER) to the plasma membrane. In a similar approach, Li et al. used mass spectrometry to identify small hydrophobic molecules released from affinity-purified yeast proteins by methanol extraction [20]. In total, 124 proteins were analyzed, including 103 kinases and 21

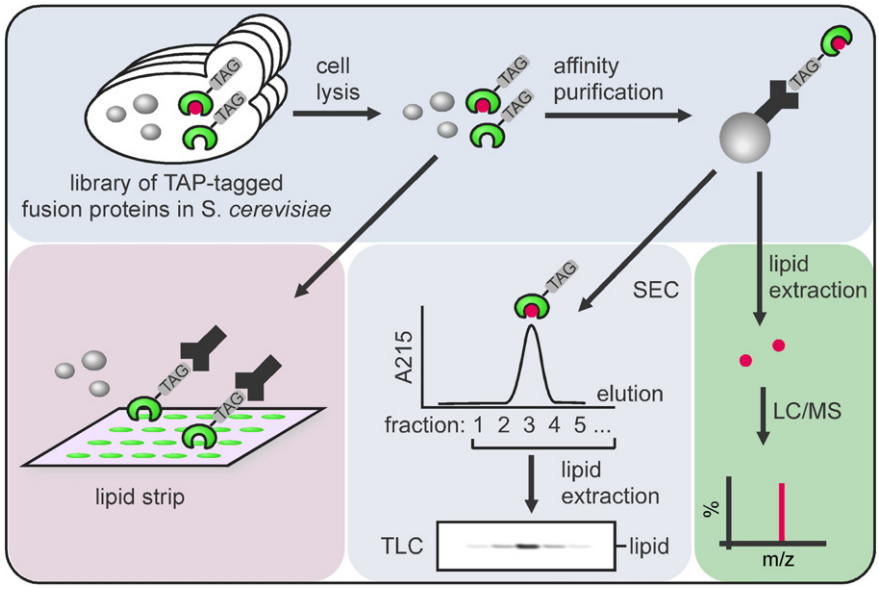

Fig. 1. Approaches for high-throughput profiling of cytoplasmic lipid–protein interactions. Cell lysates prepared from a library of yeast strains expressing TAP-tagged fusion proteins are incubated with an array of lipids bound to a nitrocellulose membrane. Interactions between the tagged proteins and the immobilized lipids are detected by immunostaining. Alternatively, TAP-tagged proteins are affinity-purified, subjected to size exclusion chromatography (SEC) and the protein-bound lipids extracted for analysis by thin layer chromatography (TLC) or LC/MS.