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Peripheral membrane proteins: Tying the knot between experiment and computation☆

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ABSTRACT

Experimental biology has contributed to answer questions about the morphology of a system and how molecules organize themselves to maintain a healthy functional cell. Single-molecule techniques, optical and magnetic experiments, and fluorescence microscopy have come a long way to probe structural and dynamical information at multiple scales. However, some details are simply too small or the processes are too short-lived to detect by experiments. Computational biology provides a bridge to understand experimental results at the molecular level, makes predictions that have not been seen *in vivo*, and motivates new fields of research. This review focuses on the advances on peripheral membrane proteins (PMPs) studies; what is known about their interaction with membranes, their role in cell biology, and some limitations that both experiment and computation still have to overcome to gain better structural and functional understanding of these PMPs. As many recent reviews have acknowledged, interdisciplinary efforts between experiment and computation are needed in order to have useful models that lead future directions in the study of PMPs. We present new results of a case study on a PMP that behaves as an intricate machine controlling lipid homeostasis between cellular organelles, Osh4 in yeast *Saccharomyces cerevisiae*. Molecular dynamics simulations were run to examine the interaction between the protein and membrane models that reflect the lipid diversity of the endoplasmic reticulum and trans-Golgi membranes. Our study is consistent with experimental data showing several residues that interact to smaller or larger extent with the bilayer upon stable binding (~200 ns into the trajectory). We identified PHE239 as a key residue stabilizing the protein–membrane interaction along with two other binding regions, the ALPS-like motif and the β6–β7 loops in the mouth region of the protein. This article is part of a Special Issue entitled: Membrane Proteins edited by J.C. Gumbart and Sergei Noskov.

1. Introduction

This review focuses on the experimental as well as computational techniques that, combined, have contributed to the understanding of the types and functions of peripheral membrane proteins (PMPs). These proteins interact strongly with lipid membranes and are influenced by the membrane lipid composition [1,2]. Proper membrane lipid composition is clearly needed for the good health and function of each organelle in eukaryotic cells [1,2]. The control mechanisms for lipid mixture conservation at each organelle are not yet fully understood, but organelle function would not be attainable without unique lipid organelle composition [2]. In fact, unbalanced lipid proportions are directly linked to several diseases like atherosclerosis and type II diabetes [3]. Lipids are usually produced at the endoplasmic reticulum (ER) and transported to the plasma membrane (PM) or trans-Golgi network (TGN) by means of vesicular transport. However, alternate routes for lipid transport have been proposed since the late 1960s [1,3]. These non-vesicular routes take place through PMP sensors and transporters tailored to work at specific regions of the cell. In addition, the interplay between transmembrane proteins and PMPs regulates and

Abbreviations: ABPP, activity-based protein profiling; ALPS, amphipathic lipid packing sensor; CERT, ceramide transport protein; CG, coarse-grained; DESRES, D.E. Shaw research; DMS, DESRES molecular structure; EM, electron microscopy; EPR, electron paramagnetic resonance; ER, endoplasmic reticulum; ER–GC, endoplasmic reticulum–Golgi complex; ERG, ergosterol; ERMES, endoplasmic reticulum–mitochondria encounter structure; ESR, electron spin resonance; F-BAR, F-Bin–Amphiphysin–Rvs; FFAT, two phenylalanines, FF, and one acidic tract; FRET, fluorescence resonance energy transfer; GFP, green fluorescent protein; GLTP, glycolipid transfer protein; HMMM, highly mobile membrane-mimetic; ITC, isothermal titration calorimetry; LP, lipases; LTP, lipid transport protein; MCS, membrane contact site; MD, molecular dynamics; NMR, nuclear magnetic resonance; OPM, orientation of proteins in membranes; OSBP, oxysterol binding protein; Osh, oxysterol binding protein homologue; PBC, periodic boundary conditions; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PH, pleckstrin homology; PI, phosphatidylinositol; PIP, phosphoinositide; PL, phospholipases; PM, plasma membrane; PMP, peripheral membrane protein; PS, phosphatidylserine; RE, replica exchange; REST, replica exchange with solute tempering; SMD, steered molecular dynamics; SPR, surface plasmon resonance; StART, steroidogenic acute regulatory transfer; TGN, trans-Golgi network; VAMP, vesicle-associated membrane protein; VAP, VAMP-associated proteins.

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maintains organelle lipid composition [1,2]. Advances and challenges in the study of PMPs are discussed here using two approaches that complement each other, experiment and computation. Common experimental techniques used in the study of PMPs are presented after a brief introduction to different PMP families, their function, and location of operation in the cell. This review focuses on the most common binding domains so far identified, and how these have led or motivated studies of other PMPs based on homology or PMPs binding to organelle(s) for their function. Some studies have focused on the study of amphipathic helices, both as stand-alone peptides as well as portions of larger proteins, we simply mentioned a few for the reader's reference but have not included a detailed summary of these in this review. The concept of membrane contact sites (MCS) is also introduced as it pertains to PMPs; and lipid transport proteins (LTPs), a subgroup of PMPs, are reviewed towards the end with a short case study on the oxysterol binding protein homologue Osh4 in yeast *S. cerevisiae*.

To expand and motivate the knowledge gained through experiments, computational techniques are discussed to show their application in the study of PMPs that transiently bind to membranes. The advances in hardware and sampling methods have indeed allowed computations to overcome some of the time and size limitations, (discussed in detail later in the review); nonetheless, both experiments and theoretical studies are required to fully understand PMP functional mechanisms and binding to the surface of the membrane. In fact, insights from simulations have motivated experiments and led the way in the study of certain phenomena. Given the long names of some PMP families, this review uses conventional acronyms that are listed separately for reference to the reader.

2. Peripheral membrane proteins (PMPs)

PMPs are classified based on their function, and most act at the lipid–water interface. They are fully soluble in water and interact with a bilayer reversibly through structural domains, electrostatic interactions, non-specific hydrophobic interactions, or using a cascade of binding events of other cytosolic proteins [4]. Despite the variety in shape and size of PMPs, reversible association with membranes is commonly dictated by the same features; namely, membrane lipid environment (rafts or charged lipids), ions like Ca^{2+}, and the polarity and geometry of the protein itself [4]. Bilayer asymmetry in organelles also plays an important role in these interactions, especially at the PM and TGN cytosolic interfaces where charged lipids are key to several processes [5]. Although these proteins are used by the cell for various functions [6–8], little is known about their mechanisms because they only bind transiently to a membrane, making it difficult to assess binding conformations and specific details of their function [7,9].

MCS are defined as regions where two membranes are 10–30 nm apart without fusing [10]. These bilayer contacts have been studied the past decade and can be facilitated by PMPs. They were first mentioned by Copeland and Dalton in the late 1950s, who studied MCS between the ER and mitochondria using early electron microscopy (EM) analyses [11], now known as the ER–mitochondria encounter structure (ERMES) [12]. Integral membrane proteins and PMPs can interact in these regions during signaling cascades or to transfer lipids between organelles *via* non-vesicular processes. PMPs can also act as tethers in such cases, simply facilitating the formation of MCS without direct participation in cargo transport [12]. Large proteins, made of thousands of amino acids, can occupy the space between MCS; they contain flexible unstructured domains that can unfold to allow interaction of the protein with two bilayers; in addition, some PMPs form dimers to accomplish their function, and do in fact need between 10 and 30 nm to operate [11,13,14]. Lipids and proteins contribute to the formation of MCS through lateral diffusion in response to concentration gradients or charge changes on the membrane surface. However, there is no detailed understanding on how these MCS form or how they are regulated [11].

The ER is by far the organelle most associated with MCS; common MCS under study are the ERMES, nucleus–vacuole junctions, ER–PM sites, and ER–GC (Golgi complex) sites [11]. Different proteins concentrate at these regions according to the lipid environment and enzymes present; *i.e.* MCS could potentially play a relevant role in lipid homeostasis by facilitating transport of the recently synthesized lipid to an opposite membrane through non-vesicular transport. In addition, more recent studies show that MCS respond to physiological changes in the cell; thus, could also be critical for cell health and homeostasis [11,12]. Known proteins at MCS include the VAP family (VAMP-associated proteins) [15]; members of the OSBP (oxysterol binding protein) family and their homologues (Osh*n* proteins with $n = 1$–7) in yeast; and members of the synaptotagmin-like-mitochondrial-lipid binding protein family, a subgroup of the tubular lipid-binding superfamily of proteins [11].

Common types of PMPs are phospholipases (PL) and lipases (LP); other relevant PMPs characterized by their structural domains include the annexin family and proteins that share the GLA-domain (involved in blood coagulation processes) [4]. PLs are enzymes used for phospholipid catabolism; different PLs cleave phospholipids at different sites, such as before or after the phosphate group and after the glycerol group at either fatty acid tail. Their function is tightly linked to substrate accessibility, *i.e.* they operate more easily at interfaces where the bilayer is more loosely packed [4]. LPs are triglycerides hydrolases that play crucial roles in lipid metabolism and only become active at membrane surfaces. These proteins have a flap or lid that blocks the active site and keeps the substrate locked in place to accomplish its purpose. Existence of the flap domain is mandatory for enzyme activation, and its movement has been proposed as a critical step in enzyme activation [4].

2.1. Lipid transport proteins (LTPs)

Yet another type of PMPs that received more attention in the past years are the LTPs, identified as key players in non-vesicular transport [16]. Non-vesicular transport mechanisms have been studied for the past thirty years, but substantial progress has been made in the past fifteen years with the emergence of more accurate experimental techniques and the rapid growth of computational biology [1,4]. Several studies show that lipid transport from the ER to the PM, TGN, and mitochondria is not impaired when vesicular transport is blocked, suggesting non-vesicular pathways are readily available in the cell [10,12,17]. Lipids like sterols, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylserine (PS) have been shown to be transported by this method, but the extent and precise mechanism of their transport is still unknown [2]. LTPs shield a lipid from the hydrophilic environment outside the bilayer during transport. A comprehensive review by Lev [16] summarizes the role of LTPs in different organelles of the cell as well as proposed mechanisms for their operation. These mechanisms are currently under study, and some LTPs are thought to be recruited to the surface at MCS to facilitate lipid exchange between two membranes that are within 30 nm of each other [2,18].

Lipids themselves dictate, to some extent, the type of LTP that interacts with them; it is in fact the specificity of a given protein for different lipid platforms and membrane environments (pH, charge, *etc.*) that maintains directionality of lipid transport [19]. Holthuis and Menon present a detailed summary of LTPs that act on the secretory pathway organelles of eukaryotic cells [2]; the most characterized lipid transporter is CERT (ceramide transport protein) and the StART (steroidogenic acute regulatory transfer) protein for sterol transport [2,20]. LTPs follow a general mechanism of embedding into the cytosolic leaflet of an organelle when they approach it in an open conformation; some may act as lipid sensors instead of transporters, and even bind two membranes simultaneously [10]. However, several LTP families are yet to be fully characterized, and their functions have been expanded beyond lipid transport due to the conservation of protein domains related to cell

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