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Plasma membrane reorganization: A glycolipid gateway for microbes ☆

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ABSTRACT

Ligand–receptor interactions, which represent the core for cell signaling and internalization processes are largely affected by the spatial configuration of host cell receptors. There is a growing piece of evidence that receptors are not homogeneously distributed within the plasma membrane, but are rather pre-clustered in nanodomains, or clusters are formed upon ligand binding. Pathogens have evolved many strategies to evade the host immune system and to ensure their survival by hijacking plasma membrane receptors that are most often associated with lipid rafts. In this review, we discuss the early stage molecular and physiological events that occur following ligand binding to host cell glycolipids. The ability of various biological ligands (e.g. toxins, lectins, viruses or bacteria) that bind to glycolipids to induce their own uptake into mammalian cells by creating negative membrane curvature and membrane invaginations is explored. We highlight recent trends in understanding nanoscale plasma membrane (re-)organization and present the benefits of using synthetic membrane systems. This article is part of a Special Issue entitled: Nanoscale membrane organisation and signalling.

1. Introduction

Ever since the discovery of the fluid mosaic model of the plasma membrane architecture [1], there has been an increasing interest in this very important part of the cell, without which the cellular machinery would fail completely. The significance of the plasma membrane is no longer restrained to a mere semipermeable barrier that functions to exchange ions and nutrients. Over the years, its involvement in several cell survival processes has been emphasized. The immunological highlight is that pathogens or their products need to fight their way into the cell by crossing this barrier. In this review, we focus on the first molecular events materializing at the outer leaflet of mammalian cell plasma membranes during microbial invasion, that translate into changes in the cytosolic facet of the plasma membrane, further advancing with a bird's eye view on the immediate downstream signaling processes preceding internalization. We seek to give a detailed account of proteinaceous ligands, representative of simplest (toxins, lectins, viruses) and complex (bacteria) microbial world, which lead to plasma membrane redesigning. Small receptor clusters formed by dynamic assembly and disassembly of plasma membrane proteins and lipids take the center stage for host pathogen interactions. Glycolipid receptor clustering and actin remodeling are important mediators of plasma membrane dynamics in the context of pathogen or toxin entry. The initiation and completion of the pathogen internalization process depend upon a number of factors such as the nature and chemistry of the different ligands, their varied receptors, the localization and distribution of receptors, binding affinity and avidity. With the advent of artificial biomimetic systems, which serve as minimal membrane systems, we have gained a better understanding of host receptor–ligand interactions and resulting endocytic mechanisms [2].

1.1. Plasma membrane architecture: A brief overview

The plasma membrane framework is very complex and hence its understanding has undergone a sea surge of changes right from the discovery of the fluid mosaic model in 1972 [1]. There have been several theses and antitheses regarding its structure [3]. The most widely accepted model today includes that of Karnovsky and co-workers, and further refined findings of Simons and Ikonen. It represents the concept of a dynamic lipid bilayer containing more ordered and tightly packed microdomains called lipid rafts that compartmentalize cellular processes [4].

1.1.1. Lipid rafts: The signaling hub

Membrane lipid rafts are specific domains of the plasma membrane, in the size range of 2–200 nm, enriched with a special subset of proteins, lipids and signaling molecules such as Yes, Lyn, H-Ras, and G protein α subunit, to name a few [5]. These rafts serve as platforms for several

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physiologically relevant ligand–receptor interactions. While the outer leaflet comprises of cholesterol, glycolipids and GPI anchored proteins, the inner facet is made of unsaturated phospholipids and mostly caveolin. There is a vast variation in size and corresponding lifetimes of lipid rafts in physiological conditions, depending on whether they are in their native or stimulated state. The unstimulated lipid raft domains are highly dynamic, small (2–10 nm) and short-lived (1 ns–1 s). Upon receiving an external stimulus, raft-associated receptors cluster together to form stable, larger domains in the size range of 10–20 nm, which possess a longer lifetime [6]. These larger domains are formed by recruiting cholesterol and other saturated lipids near the site of ligand binding [7]. Pathogens target lipid raft components and hijack cellular signaling pathways in order to evade the host system. It is also believed that the entry of pathogens, which is mediated by lipid raft components, increases their chance to avoid lysosomal fusion and overpower the host immune response to sustain intracellular survival [8]. The importance of membrane lipid rafts in signaling has been proven by studies that showed the inhibition or stimulation of signaling pathways due to disruption of lipid rafts, e.g. by cholesterol depletion, conversion of sphingomyelin to ceramide and several other approaches, which are discussed in later sections. The recent understanding of lipid rafts and associated protein–lipid diffusion has been elaborately reviewed in Ref. [9].

1.1.2. Actin as organizer of plasma membrane architecture

Major functions of the actin meshwork include phagocytosis, macropinocytosis, endocytosis, formation of lamellipodia and filopodia. The actin cortex not only provides a structural stability to the entire cell, but also is dynamically involved in guiding cytosolic proteins such as Rho GTPases to associate with membrane receptors and transduce signaling cascades. The actin cortex acts as a barrier that moderates the rate of diffusion of proteins and lipids within the plasma membrane. Membrane blebs and artificial membranes that lack actin have a higher rate of protein and lipid diffusion when compared to the membranes that contain actin [10]. Recent studies proved that actin depolymerisation prevented the formation of lipid rafts by increasing the diffusion rate of lipid raft markers [11,12]. A multitude of weak binding interactions between cortical actin components and plasma membrane phospholipids provide the adhesion energy required to keep the membrane tethered to the cytoskeletal surface [13].

1.1.3. Membrane curvature and sorting at the plasma membrane

Membrane curvature is a phenomenon involved in several biologically significant processes such as mitosis, endocytosis, apoptosis, membrane fusion and fission. Positive or negative membrane curvatures lead to changes in the total surface area of the cell membrane. Factors that contribute to membrane curvature include shape, size, asymmetric distribution and bending rigidity of lipids and proteins. Small GTPases, clathrin, caveolin, dynamin and Bin/Amphiphysin/Rvs (BAR) domain proteins help in scaffolding the new structures formed due to membrane bending. The N-BAR domain proteins such as endophilin, amphiphysin, centaurin and nexins, sense membrane bending and adhere to these curved regions to induce 3D tubular structures of diameters ranging between 20 and 60 nm [14]. In a recent study by Linkner et al., I-BAR proteins induced membrane invaginations on fluorescent GUVs that contained $PI(4,5)P_2$. Furthermore, I-BAR proteins were observed to concentrate on constrictions of phagocytic cups, indicating their role in phagocytic uptake of pathogens [15]. Recent studies have reported a strong influence of $PI(4,5)P_2$ in the membrane deforming activity of BAR domain proteins [16].

In molecular simulation studies, it was speculated that membrane curvature may be induced by lateral heterogeneous distribution of lipids and transmembrane proteins, or it is possible that protein–lipid clusters accumulate in preformed membrane invaginations due to their heterogeneous nature [17]. The extent of curvature determines packing of lipids, which explains the looser packaging of lipids in the outer leaflet than in the inner leaflet of the plasma membrane. A recent review on membrane bending mechanisms explains the effect of membrane protein crowding and resulting uneven mass distribution across membrane bilayers [18]. Clustering of plasma membrane receptors upon ligand binding may result in similar mass distribution on cell membrane and artificial bilayer systems [17]. As an example to this phenomenon, we consider glycolipid receptor clustering upon toxin binding to a lipid bilayer (Fig. 1). It is deducible that the clustered ligand–receptor mass exerts a stress on the outer membrane leaflet at the site of toxin binding, which translates into negative membrane curvature, leading to increased surface area to facilitate additional ligand binding. As a result of this local compaction of lipids, more ligands bind, boosting the membrane deformation, leading to tubule formation once a critical concentration of ligand–receptor clusters is achieved.

1.1.4. Artificial membrane systems: Essential tools for studies on membrane dynamics

Among the biomimetic membrane models available, giant unilamellar vesicles (GUVs) and planar membrane systems like supported lipid bilayers (SLBs) have become important tools to understand membrane dynamics upon binding of proteinaceous ligands such as toxins or viruses. The artificial membranes are meant to be simplified models of the plasma membrane, which are used in place of native cellular membranes in order to prevent data overcrowding, improve data analysis and interpretation. Hence, they contain only a few main components of the plasma membrane, such as phospholipids, e.g. 1,2-dioleoylphosphatidyl choline (DOPC), cholesterol, sphingomyelin, fluorescent membrane markers (to visualise the bilayer), and a receptor molecule such as the ganglioside GM1. SLBs represent a planar membrane sitting on a solid support [19]. The main advantage of this system is its stability and hence the possibility to image in high-resolution using atomic force microscopy (AFM). By performing AFM, one could gain data on the nanoscale membrane organisation of cellular and artificial membrane systems [20–22]. In the recent past, a combination of AFM with fluorescence microscopy and spectroscopy tools has been established to study ceramide-mediated membrane rafting [23,24]. GUVs with sizes ranging from 10 to 100 μm are one of the most widely exploited models to study lipid rafts by fluorescence microscopy. The current understanding of the raft hypothesis, derived from membrane

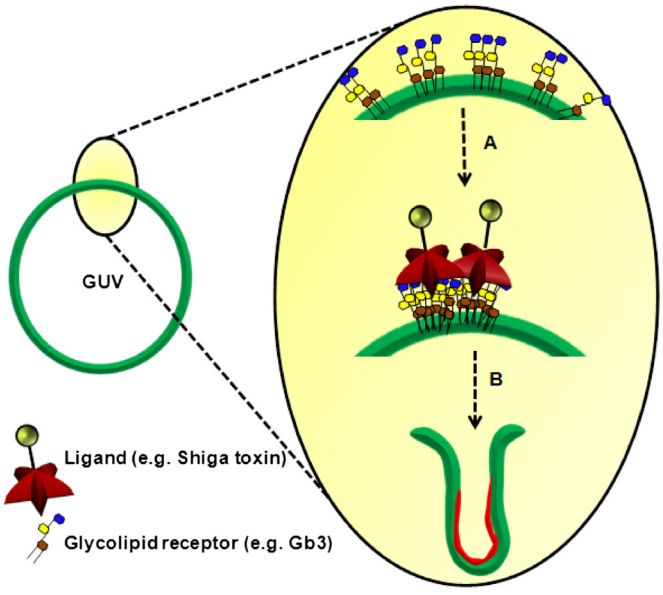

Fig. 1. Schematic illustration of ligand-driven receptor clustering and tubule formation in giant unilamellar vesicles. Glycolipids cluster upon highly specific toxin binding (A), which induces the formation of tubular membrane invaginations (B). The figure is not drawn to scale. The right panel is an enlarged view of the small, yellow shaded region of the giant unilamellar vesicle (left).

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