Biochimica et Biophysica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

Rafting through traffic: Membrane domains in cellular logistics

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ARTICLE INFO

Article history:
Received 28 May 2014
Received in revised form 28 July 2014
Accepted 31 July 2014
Available online xxxx

Keywords:
Membrane domain
Lipid raft
Subcellular traffic
Sorting
Endocytosis

ABSTRACT

The intricate and tightly regulated organization of eukaryotic cells into spatially and functionally distinct membrane-bound compartments is a defining feature of complex organisms. These compartments are defined by their lipid and protein compositions, with their limiting membrane as the function interface to the rest of the cell. Thus, proper segregation of membrane proteins and lipids is necessary for the maintenance of organelle identity, and this segregation must be maintained despite extensive, rapid membrane exchange between compartments. Thus, sorting processes of high efficiency and fidelity are required to avoid potentially deleterious mis-targeting and maintain cellular function. Although much molecular machinery associated with membrane traffic (i.e. membrane budding/fusion/fission) has been characterized both structurally and biochemically, the mechanistic details underlying the tightly regulated distribution of membranes between subcellular locations remain to be elucidated. This review presents evidence for the role of ordered lateral membrane domains known as lipid rafts in both biosynthetic sorting in the late secretory pathway, as well as endocytosis and recycling to/from the plasma membrane. Although such evidence is extensive and the involvement of membrane domains in sorting is definitive, specific mechanistic details for raft-dependent sorting processes remain elusive.

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1. Introduction

The self-organization of cellular macromolecules into structurally and functionally distinct subcellular compartments is one of the most intriguing and complex questions in biology. Most eukaryotic cell organelles are delimited by a lipid and protein membrane, which separates the cytoplasm from a topologically disconnected aqueous lumen. Because these membranes are the interface between the organelles and the rest of the cell, their molecular identities (i.e. protein and lipid composition) play a key role in defining the function of a given compartment as a whole. Therefore, the efficient and accurate sorting of membrane molecules between organelles underlies much of subcellular organization. (See Table 1.)

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http://dx.doi.org/10.1016/j.bbamem.2014.07.029
0005-2736/

Recent decades have seen the discovery and characterization of the extensive proteinaceous machinery responsible for intracellular membrane transport. One general theme is that membranes are sorted and transported by small (i.e. less than 100 nm diameter) vesicular intermediates produced by the action of a "coat protein", which are actually large, multiprotein complexes. The classic examples of such "coatomers" are the COat Protein complexes (COPI and COPII) that mediate vesicle formation and transport between the endoplasmic reticulum (ER) and early Golgi, and the polymerized clathrin cages in the late secretory pathway and endocytosis. An alternative vesicle-producing apparatus – that does not proceed via a coated intermediate – is the ESCRT machinery responsible for lumen-directed vesicle fission in endocytosis [1], virus budding [2], and membrane repair [3]. These machines are responsible for the generation and fission of transport vesicles, but all require additional factors for targeted, vectorial vesicle transport to the appropriate cellular compartment and fusion with the target membrane. These functions are served in part by the famous SNARE (Soluble N-ethylmaleimide-sensitive-factor Attachment protein REceptor) proteins [4], which determine where and when a given transport vesicle will fuse. In addition to this core machinery, hundreds of ancillary proteins regulate subcellular traffic, including the vital sorting function served by Adapter Proteins (AP1-4 [5]) to selectively recruit specific proteins into the transport carriers, and the Rab GTPases that act as "address labels" for the various organelles [6].

In contrast to the wealth of information available for subcellular *protein* sorting, a mechanistic understanding of how *lipids* are distributed in cells remains elusive. Organellar membranes have distinct lipid compositions (though clean, detailed data to this point are scant) that are likely required for their function and cannot be accounted for solely by enzymatic production/turnover [7–9]. However, how the cells achieve this steady-state heterogeneity despite active and rapid exchange of lipids between compartments remains unclear, in part because the rules for sorting lipids and proteins are quite different:

(1) lipids are not sorted absolutely, i.e. all membranes contain broadly similar lipid classes (e.g. phosphatidylcholine), but the specific molecular identities and, more importantly, relative concentrations of the lipids vary between compartments.
(2) lipids are not covalently linked into membranes, but are organized by weaker intermolecular interactions which give rise to the fluid matrix of the bilayer
(3) whereas proteins are often sorted by specific intermolecular coupling between a cargo protein and the coatomer, lipids are generally too numerous and too small for such one-by-one selection (although there are important exceptions [10–12]).

Because of these unique features, specific mechanisms are required for intracellular lipid sorting. Membrane lipid monomer transport through the cytoplasm does not occur on cell-relevant time scales (though cholesterol transport may be an exception [13]), because despite lipids not being covalently incorporated into the bilayer, the entropic penalty for hydrating their aliphatic regions makes spontaneous diffusion of lipid monomers into the cytoplasm extremely unfavorable [14]. In some cases, carrier proteins facilitate monomeric lipid transport by providing a hydrophobic cavity [10–12]. Additionally, physical contact sites between the ER and other organellar membranes may provide channels for direct lipid transfer between organelles [15]. However, these mechanisms are insufficient for the whole lipidome sorting observed in subcellular organelles; thus, membrane lipids, like proteins, are generally trafficked by vesicular intermediates, begging the question of how such vesicles "choose" the lipids destined for a particular compartment.

Lateral membrane domains of distinct compositions provide an ideal platform for lipid sorting. The archetype of such domains are membrane rafts, defined as lipid and protein assemblies whose formation is dependent on the preferential interactions between specific lipids (sterols, glycosphingolipids, and saturated lipids) that drive the formation of a liquid-ordered membrane state that coexists with a relatively disordered state as fluid, lateral domains [16]. It is important to emphasize that the conception of rafts as long-lived, large, stable domains is imprecise and probably incorrect — rafts in the PM are believed to be small and highly dynamic [17], and there remains active controversy about the physical properties, compositions, and mechanistic consequences of raft domains. However, several recent observations – most notably, microscopically observable liquid–liquid phase separation in biological membranes [18–22] – have provided strong evidence for their existence and biological relevance. Moreover, the proposed size of such domains (tens to hundreds of nm [17]) and their capacity to sequester both lipids and proteins provide rafts with ideal features for acting as sorting mechanisms in membrane trafficking.

This review focuses on evidence supporting the central role of raft domains in subcellular membrane sorting. We start with a discussion of the genesis of the raft hypothesis to explain the distinct membrane compositions of apical and basolateral plasma membrane (PM) domains in polarized epithelia, and expand on the general utilization of these domains in secretory sorting and trafficking to the PM in both polarized and non-polarized cells. Next, we summarize the robust literature describing raft domains in endocytosis and sorting in the endocytic system, before briefly reviewing the scant information on the protein machinery that supports raft-mediated trafficking. As with much of the raft field, there remain more questions than answers about the mechanistic details of how these domains mediate trafficking; however, this review is intended to provide a primer to the topic, while highlighting the abundant evidence in support for the hypothesis that rafts are a key mediator of the specific membrane compositions of several subcellular organelles.

2. Rafts in secretory traffic

The Golgi Apparatus (GA) is the intermediate between the site of most lipid synthesis (the endoplasmic reticulum — ER) and other membrane-bound organelles, thus making it a major membrane sorting station in the cell. More specifically, while the "cis" portions of the GA (i.e. those associated with bi-directional membrane exchange with the ER) are responsible for the post-translational modification and refinement of membrane proteins, the most "trans" cisterna of the organelle, termed the trans-Golgi network (TGN), is the site of selection for export of membrane (and luminal) components via newly assembled transport carriers destined for their final cellular location (Fig. 1). In some cases (e.g. the mannose-6-phosphate receptor for lysosomal delivery [23]), the sorting determinants are simple and clear. However, for most membrane lipids and proteins, the mechanisms of distribution remain unresolved. In epithelial cells, this problem is further complicated by the existence of a highly specialized plasma membrane domain, the apical PM, coexisting contiguously with a basolateral PM. These domains must be compositionally distinct because their functional requirements are very different: the apical PM must often provide a robust barrier between the cell and a harsh and inhospitable environment (e.g. gut or kidney lumen), whereas the basolateral is responsible for exchanging information and nutrients with the rest of the body.

2.1. Genesis of the raft hypothesis

An early observation in epithelial cell biology was that not only the protein, but also the lipid composition of the apical PM is highly differentiated from the basolateral, most notable in the enrichment of glycosylated sphingolipids (GSLs) and cholesterol, and relative depletion of glycerophospholipids (see studies cited in Ref. [24]). The sorting of both glycosylated proteins [25] and lipids [26] to the apical surface seemed to occur simultaneously in the TGN, suggesting a membrane-mediated mode of action, which was confirmed by the isolation of distinct vesicle subtypes originating at the TGN and containing either apical or basolateral cargo [27]. To explain these observations, a model based on the known propensity for sphingolipids to self-associate via

Please cite this article as: B. Diaz-Rohrer, et al., Rafting through traffic: Membrane domains in cellular logistics, Biochim. Biophys. Acta (2014), http://dx.doi.org/10.1016/j.bbamem.2014.07.029