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Review

Membrane microdomains in immunoreceptor signaling

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ABSTRACT

Membrane microdomains denoted commonly as lipid rafts (or membrane rafts) have been implicated in T-cell receptor (TCR), and more generally immunoreceptor, signaling for over 25 years. However, this area of research has been complicated by doubts about the real nature (and even existence) of these membrane entities, especially because of methodological problems connected with possible detergent artefacts. Recent progress in biophysical approaches and functional studies of raft resident proteins apparently clarified many controversial aspects in this area. At present, the prevailing view is that these membrane microdomains are indeed involved in many aspects of cell biology, including immunoreceptor signaling. Moreover, several other types of raft-like microdomains (perhaps better termed nanodomains) have been described, which apparently also play important biological roles.

1. Introduction

The concept of membrane microdomains has been formulated already in 1982 [1] and reflected the experimental evidence for membrane lateral heterogeneity observed by various biophysical techniques in model membrane systems as well as in native cell membranes. It was suggested that membrane lipids can undergo phase separations, interact more or less selectively with membrane proteins and with submembrane cytoskeletal elements. Later, a strong biochemical indication of heterogeneity of biological membranes was based on selective resistance of certain membrane proteins to solubilization by some detergents, e.g. Triton X100, Brij-series, NP-40 or CHAPS.

The detergent-resistant membrane microdomains (DRMs) started to be called lipid rafts [2] and for some time these terms were considered as more or less synonymous. These entities became especially interesting for immunologists when it was found that they contain several important signaling molecules involved in immunoreceptor signaling [3]. For years, lipid rafts (more correctly membrane rafts, as they are not composed solely of lipids) of immunocytes and other cell types were defined (mostly based on the results of biochemical experiments involving detergent-resistance) as membrane microdomains enriched in glycosphingolipids and glycerolipids containing mainly saturated fatty acid residues, cholesterol and lipid-modified proteins, including especially the glycosylphosphatidylinositol (GPI)-anchored ones.

Studies on artificial membrane systems indicated that these membrane microdomains are held together mainly by hydrophobic interactions between saturated fatty acid residues of their main lipid constituents and further stabilized by cholesterol molecules, which are in biological membranes intercalated between bulky glycolipids [4]. This particular lipid mixture may form a specific "ordered liquid phase", the physical properties of which are different from the rest of the plasma membrane. Treatments of membranes with cholesterol-depleting agents [5], cholesterol-modifying enzymes or biosynthetic replacement of saturated fatty acid residues in their sphingolipids by unsaturated ones [6] were found to destabilize the rafts so they lost their detergent resistance.

Later it became obvious that the use of detergents may produce more or less significant artefacts – the composition and properties of the DRMs were clearly dependent on the chemical nature and concentration of the detergent, temperature and duration of the solubilization (see below). Thus, DRMs generally should not be equated with native rafts; some authors even doubted about the very existence of raft microdomains in native membranes. Many studies aimed to demonstrate the existence and properties of the raft microdomains in more or less native biological membranes. An obvious approach has been based on the use of microscopic methods. These are however of limited use because the size of these microdomains appears to be in most cases under the resolution limit of conventional optical microscopy. Nevertheless, the use of lipid and protein probes preferentially incorporating into membrane areas enriched in certain types of lipids confirmed the lateral heterogeneity of not only artificial, but also native biological membranes [7–9].

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A specific type of raft-like microdomains are caveoli, morphologically distinct plasma membrane invaginations stabilized by cholesterol-binding protein caveolin and therefore readily observable by electron microscopy [10].

A major advance in the studies of raft microdomains in relatively native cell membranes was the introduction of the techniques producing plasma membrane vesicles. In this system, phase separation of distinct lipid-based membrane domains can be observed and conditions affecting this process and its biological implications can be studied under relatively natural conditions. The microscopically observable membrane domains observed in these studies apparently arise due to spontaneous coalescence of dynamic "elementary membrane rafts" present under physiological conditions [11]. Importantly, in agreement with previous biochemical, detergent-based experiments, different membrane proteins are selectively segregated into such relatively native raft membrane domains, often based on their palmitoylation status.

Another powerful approach to study the role of raft microdomains in physiological functions of proteins residing there is the use of mutants targeted outside of rafts. This has been achieved mostly by modifications altering the palmitoylation status of such proteins (see below). A very telling example of importance of targeting of biologically active molecules into raft microdomains was demonstrated by Simons and colleagues [12]; raft-targeted inhibitors of a raft-associated enzyme were markedly more active than those targeted outside rafts.

The currently widely accepted idea about membrane rafts is that they are tiny, very dynamic "islets" (only tens of nanometers in size), containing a few hundreds of lipid molecules and mostly single protein molecules, spontaneously formed as a result of phase transitions in complex mixtures of membrane lipids and proteins, that cannot be easily observed on intact cell surface by existing microscopic techniques. These "elementary rafts" can be often stabilized to form larger, readily observable membrane domains following various physical or chemical perturbations affecting the protein-protein or protein-lipid interactions [13]. The properties of raft microdomains in native cell membranes may be strongly influenced by interactions with submembrane skeleton and cytoskeleton. One so far unresolved problem is to what extent there is a coupling of the raft microdomains of both membrane leaflets (i.e. the external and cytoplasmic leaflet in the case of plasma membrane). It can be speculated that such coupling may be mediated by specific "raftophilic" transmembrane proteins, such as the palmitoylated transmembrane adaptor proteins mentioned below.

2. Involvement of lipid rafts in immunoreceptor signaling

The existence of a possible relationship between T cell activation and membrane rafts became apparent when it was realized that T cells can be activated by antibody-mediated cross-linking of surface glycoproteins such as Thy-1 or Ly-6. This was somewhat mysterious, as these molecules apparently cannot directly communicate with cytoplasmic signaling molecules, because they are entirely extracellularly oriented and anchored in the external plasma membrane leaflet by means of a covalently attached glycolipid moiety, glycosylphosphatidylinositol (GPI). Cross-linking of numerous GPI-anchored proteins (and also glycolipids) results in cellular responses strikingly similar to those elicited by immunoreceptors, such as T-cell receptor (TCR), B-cell receptor (BCR) or some Fc-receptors (FcR). These observations could be rationalized by the fact that GPI-anchored proteins are components of membrane rafts, which contain several key signaling components involved also in immunoreceptor signaling (Src-family kinases (SFKs), transmembrane adaptor proteins, phosphatidylinositol bis-phosphate (PIP2), G-proteins). Importantly, T-cell activation via cross-linking of GPI-anchored proteins was found to be dependent on expression of TCR ζ chain. Therefore a plausible model was that cross-linking of GPI-anchored proteins (or raft glycolipids) results in partial co-cross-linking of TCR and possible mimicking of early steps in physiological TCR activation (reviewed in [14]). However, a question remained whether this is just an experimental artefact, or if this is an "informative artefact" and membrane rafts are actually involved in physiological activation of signaling cascades initiated by immunoreceptors. Indeed, biochemical studies in several types of immunocytes revealed that experimental cross-linking of the respective immunoreceptors (TCR, BCR, FcR) is accompanied by association of the receptors with DRMs, i.e. presumably membrane rafts. Thus, also cross-linking by their natural ligands may induce their functionally relevant merging with membrane rafts. As a result, the tyrosine based-activation motifs (ITAM) in cytoplasmic tails of immunoreceptor complexes (CD3, ζ-chain, Ig-α,β FcR γ-chain) become exposed to SFKs present in the rafts. Phosphorylated ITAMs of these signaling chains then serve as docking sites for Syk family kinases (ZAP70 or Syk). Activated ZAP70 in T cells phosphorylates another membrane raft component – the transmembrane adaptor protein LAT ("linker for activation of T cells"), resulting in its association with several other cytoplasmic signaling proteins, including phospholipase Cγ1 (PL Cγ1). This promotes further steps in the TCR-induced signaling cascades. Importantly, also TCR co-receptors, CD4 and CD8, are palmitoylated proteins associated with membrane rafts. Therefore, their association with TCR after contact of the T cell with antigen presenting cells (APC) may contribute to co-aggregation of the receptor complex with membrane rafts. Alternatively, TCR (and other immunoreceptors) may be pre-associated with membrane rafts [15] and its ligation just reorganizes somehow this assembly to allow for optimal exposure of the CD3 and ζ chains to the SFKs.

The importance of membrane rafts immunoreceptor signaling is supported by findings that palmitoylation-deficient mutants of several of the raft resident proteins such as SFKs, CD8β, pre-TCR or LAT [16–20] are excluded from the rafts which results in functional defects. A fraction of a negative regulator of Src-family kinases activity, the protein tyrosine kinase (PTK) Csk, is also found in membrane rafts, due to its association with the phosphorylated transmembrane adaptor protein PAG ("phosphoprotein associated with GEMs") also called Cbp ("Csk binding protein") [21,22], a palmitoylated membrane raft resident molecule. Cross-linking of TCR on resting αβT cells causes rapidly a transient dephosphorylation of PAG accompanied by Csk dissociation. This in turn contributes to increased SFK (Lck, Fyn) activity needed for TCR signaling, because the negative regulator of these SFKs is now removed from their vicinity. On the other hand, protein kinase A type I, which also associates with membrane rafts of activated T cells, activates by phosphorylation the raft-associated Csk and thereby contributes to inhibition of SFKs [23]. Another raft-associated transmembrane adaptor, LIME ("Lck interacting molecule"), becomes tyrosine phosphorylated and Csk-associated after cross-linking of the CD4 or CD8 co-receptors [24]; however, the biological importance of this effect is not clear because the LIME gene knock-out apparently does not have any defects in TCR signaling.

The major costimulatory receptor of T cells, CD28, is present in the non-raft part of the resting T cell membrane, but after activation-induced cross-linking it may relocate to rafts [25]. The major negative regulator of T cell activation and CD28 competitor, CTLA-4 (CD152), was reported to be constitutively associated with membrane rafts of activated T cells and may interfere with relocation rafts activated T cell plasma membrane [26].

Among other important raft-associated signaling molecule of activated T cell are the scaffolding protein CARMA1 and protein kinase Cθ. The former molecule is the critical regulator of TCR-induced NF-κB activation [27], while the latter cytoplasmic

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