Structure of presentations

Introduction : **5-10** min short review of the theme in respect of the historical period

Aim of the study : **2 min**

Methods used : **5-10** min

Results: **15 min**

Conclusion **3 min**

Take home messages 2 min

Week-1 : In the membrane

A classic review review for background :

Revisiting the fluid mosaic model of membranes, Jacobson et al Science 1995

DOI: 10.1126/science.7770769

Modern reviews :

The mystery of membrane organization: composition, regulation and roles of lipid rafts.

Erdinc Sezgin, Ilya Levental, Satyajit Mayor, Christian Eggeling

Nature Reviews Molecular Cell Biology **volume 18**, pages 361–374 (2017)

https://doi.org/10.1038/nrm.2017.16

NB have a look at the supplements.

Membrane organization and lipid rafts

Kai Simons [1], Julio L Sampaio Cold Spring Harb Perspect Biol

2011 Oct 1;3(10):a004697.

doi: 10.1101/cshperspect.a004697.

Week-1 : In the membrane Presenting on Nov-26

Group 1- Louise DROIN - Romain GUICHONNET - Flavia ZABALA PEREZ Laure CARMINATI

Phospholipids undergo hop diffusion in compartmentalized cell membrane

Takahiro Fujiwara 1 , Ken Ritchie, Hideji Murakoshi, Ken Jacobson, Akihiro Kusumi

Journal of Cell Biology July 2002; 157(6):1071-81. ***DOI:10.1083/jcb.200202050***

PMID: 12058021 PMCID: PMC2174039 **DOI: 10.1083/jcb.200202050**

Abstract

The diffusion rate of lipids in the cell membrane is reduced by a factor of 5-100 from that in artificial bilayers. This slowing mechanism has puzzled cell biologists for the last 25 yr. Here we address this issue by studying the movement of unsaturated phospholipids in rat kidney fibroblasts at the single molecule level at the temporal resolution of 25 micros. The cell membrane was found to be compartmentalized: phospholipids are confined within 230-nm-diameter (phi) compartments for 11 ms on average before hopping to adjacent compartments. These 230-nm compartments exist within greater 750-nm-phi compartments where these phospholipids are confined for 0.33 s on average. The diffusion rate within 230-nm compartments is 5.4 microm2/s, which is nearly as fast as that in large unilamellar vesicles, indicating that the diffusion in the cell membrane is reduced not because diffusion per se is slow, but because the cell membrane is compartmentalized with regard to lateral diffusion of phospholipids. Such compartmentalization depends on the actin-based membrane skeleton, but not on the extracellular matrix, extracellular domains of membrane proteins, or cholesterol-enriched rafts. We propose that various transmembrane proteins anchored to the actin-based membrane skeleton meshwork act as rows of pickets that temporarily confine phospholipids.

Group 2 - Coralie Reuse, Coralie Charpié, Jonas Richard, Vera Dias Gomes.

Sharma, P. et al. Nanoscale organization of multiple GPI-anchored proteins in living cell membranes.

***Cell* 116, 577–589 (2004). The first demonstration of cholesterol-assisted nanoscale clusters in living cells.**

doi: 10.1016/s0092-8674(04)00167-9.

Abstract

Cholesterol and sphingolipid-enriched “rafts” have long been proposed as platforms for the sorting of specific membrane components including glycosyl-phosphatidylinositol-anchored proteins (GPI-APs), however, their existence and physical properties have been controversial. Here, we investigate the size of lipid-dependent organization of GPI-APs in live cells, using homo and hetero-FRET-based experiments, combined with theoretical modeling. These studies reveal an unexpected organization wherein cell surface GPI-APs are present as monomers and a smaller fraction (20%–40%) as nanoscale (<5 nm) cholesterol-sensitive clusters. These clusters are composed of at most four molecules and accommodate diverse GPI-AP species; crosslinking GPI-APs segregates them from preexisting GPI-AP clusters and prevents endocytosis of the crosslinked species via a GPI-AP-selective pinocytic pathway. In conjunction with an analysis of the statistical distribution of the clusters, these observations suggest a mechanism for functional lipid-dependent clustering of GPI-APs.

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Group 3 - Ami De Carli , Aldric Mercier , Emile Chauvel

2 linked papers

Direct chemical evidence for sphingolipid domains

in the plasma membranes of fibroblasts.

Jessica F. Frisz, Kaiyan Lou, Haley A. Klitzing, +8 , and Mary L. Kraft

Proc Nat Acad Sci USA 110,

https://doi.org/10.1073/pnas.1216585110

Abstract

Sphingolipids play important roles in plasma membrane structure and cell signaling. However, their lateral distribution in the plasma membrane is poorly understood. Here we quantitatively analyzed the sphingolipid organization on the entire dorsal surface of intact cells by mapping the distribution of ^{15}N-enriched ions from metabolically labeled ^{15}N-sphingolipids in the plasma membrane, using high-resolution imaging mass spectrometry. Many types of control experiments (internal, positive, negative, and fixation temperature), along with parallel experiments involving the imaging of fluorescent sphingolipids—both in living cells and during fixation of living cells—exclude potential artifacts. Micrometer-scale sphingolipid patches consisting of numerous ^{15}N-sphingolipid microdomains with mean diameters of ~200 nm are always present in the plasma membrane. Depletion of 30% of the cellular cholesterol did not eliminate the sphingolipid domains, but did reduce their abundance and long-range organization in the plasma membrane. In contrast, disruption of the cytoskeleton eliminated the sphingolipid domains. These results indicate that these sphingolipid assemblages are not lipid rafts and are instead a distinctly different type of sphingolipid-enriched plasma membrane domain that depends upon cortical actin.

Sphingolipid domains in the plasma membranes of fibro blasts are not enriched with cholesterol -

Jessica F Frisz [‡], Haley A Klitzing [‡], Kaiyan Lou [§], Ian D Hutcheon [¶], Peter K Weber [¶], Joshua Zimmerberg [‖], Mary L Kraft

J Biol Chem

2013 Apr 22;288(23):16855–16861. **doi: 10.1074/jbc.M113.473207**

Abstract

The plasma membranes of mammalian cells are widely expected to contain domains that are enriched with cholesterol and sphingolipids. In this work, we have used high-resolution secondary ion mass spectrometry to directly map the distributions of isotope-labeled cholesterol and sphingolipids in the plasma membranes of intact fibroblast cells. Although acute cholesterol depletion reduced sphingolipid domain abundance, cholesterol was evenly distributed throughout the plasma membrane and was not enriched within the sphingolipid domains. Thus, we rule out favorable cholesterol-sphingolipid interactions as dictating plasma membrane organization in

fibroblast cells. Because the sphingolipid domains are disrupted by drugs that depolymerize the cells actin cytoskeleton, cholesterol must instead affect the sphingolipid organization via an indirect mechanism that involves the cytoskeleton.