Detection and characterization of rafts can be made via microscopy in case that their size is within the resolution of the microscope, but in case that microscopy is combined with spectroscopic data, even nanodomains can be detected (de Almeida et al., 2007). Also from time-resolved data and FRET modeling, information about their size can be obtained (de Almeida et al., 2005), and the detection of nanosize structures is unequivocal.

Ceramide (Cer) is a lipid related to several biological processes such as apoptosis, and rafts are closely related to Cer, since it is the precursor of complex sphingolipids, and can also be formed in the plasma membrane via enzymatic hydrolysis of SM, one of the canonical rafts constituents. Before studying the interaction of rafts and Cer it was necessary to carry out a detailed characterization of the phases that Cer can form in a fluid phospholipid matrix, and it was observed that small amounts of Cer (either C16:0, 4% (Silva et al., 2006), or C24:1, 10%) can induce the formation of gel phases. In the presence of Cer, SM, and POPC, it was found that Cer recruits POPC and PSM in the fluid phase to form extremely ordered and compact gel domains. Gel domain formation by low Cer mol fraction (up to 12 mol.%) is enhanced by physiological SM levels (~20–30 mol.% total lipid). For higher SM content, a three phase situation, consisting of fluid (POPC-rich)/gel (SM-rich)/gel (Cer-rich) coexistence is obtained. To determine the fraction of each phase a quantitative method was developed, which allowed establishing the complete ternary phase diagram (Castro et al., 2007). This helps to predict Cer-rich gel domain formation, and explains its enhancement through SM/Cer interactions.

To understand the interplay of ceramide with lipid rafts, the previous mixture, but now containing Chol was studied, and revealed that low Cer concentrations strongly change both the biophysical properties and lipid lateral organization of the ternary mixtures in the low-to-intermediate Chol/SM-, small raft size range (<25 mol.% Chol). For these mixtures, Cer recruited up to three PSM molecules for the formation of very small (~4 nm) and highly ordered gel domains, which became surrounded by rafts (liquid-ordered phase) when Chol/SM content increased. However, the size of these rafts did not change, showing that Cer did not induce the formation of large platforms or the coalescence of small rafts. In the high Chol/SM-, large raft domains range (>33 mol.% Chol), Chol completely abolished the effect of Cer. Lipid rafts govern the biophysical properties and lateral organization in these last mixtures (Silva et al., 2007).

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References


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PL 3

The biology of metazoan-specific phosphatidylinositol transfer protein

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Phosphatidylinositol (PtdIns)/phosphatidylcholine (PtdCho) transfer proteins (PITPs) regulate key interfaces between lipid signaling and membrane trafficking in eukaryotes. The Sec14-like cohort of the eukaryotic PITP ensemble is defined by a large protein superfamily consisting of greater than 600 members, and these proteins are present from yeast to humans. However, there also exists a second class of PITPs whose members are structurally unrelated to the Sec14-like PITPs. These PITPs are found only in Metazoa. Such an evolutionary restriction suggests the metazoan PITPs (metPITPs) execute functions unique to the most complex biological systems, and identify the metPITPs as molecular signatures of the highest eukaryotes. I will summarize our current ideas concerning the common roles of Sec14-like and metPITPs as nanoreactors for phosphoinositide synthesis and signaling. I will also discuss our most recent progress towards elucidating the physiological functions of metPITPs in genetically tractable model systems. Particular emphasis will be devoted to our recent studies of the role of PITPs function in the mouse, and the pathologies associated with functional ablation of this protein.

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SO 1

Modulating the skin barrier function by DMSO: molecular dynamics simulations of hydrophilic and hydrophobic transmembrane pores

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The dense lipid bilayers at the outer surface of the skin represent the primary barrier to molecules penetrating the human skin. One approach to overcome this barrier, with promising applications in administering medicinal drugs to the body, is to employ chemical permeability enhancers. How these enhancers, such as dimethyl-sulfoxide (DMSO), exert their effect at the molecular level is only partly understood.

We present molecular dynamics simulations to elucidate the interaction of DMSO with bilayers of ceramide 2, the most abundant lipid in the skin. The DMSO molecules are found to weaken the impermeable crystalline bilayers, and even to cause a transition to a fluidized phase at high DMSO concentrations. This is consistent with the experimental evidence that a substantial concentration of DMSO is required to enhance the permeability of the skin.

Trans-membrane pores are created using a constraint technique, and the free energy change during pore formation is calculated. High DMSO concentrations yield archetypal hydrophilic pores, i.e. the membrane edge surrounding the pore is lined with lipid head groups, while in pure water we observe the formation of hydropho-
The plasma membrane contains nanodomains enriched in cholesterol and sphingolipids (lipid rafts) which are believed to be more ordered than the bulk membrane and play an important role in early T cell signalling. Laurdan is a UV-excitable dye that can be excited by a 364 nm laser and emits at 490 nm. We find that nanometer-sized pores are actually empty. The origins and consequences of these vapour pores are discussed.

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SO 2

Plasma membrane order in T cell signalling

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The plasma membrane contains nanodomains enriched in cholesterol and sphingolipids (lipid rafts) which are believed to be more ordered than the bulk membrane and play an important role in early T cell signalling. Laurdan is a UV-excitable dye that can be excited by a 364 nm laser and emits at 490 nm. We find that nanometer-sized pores are actually empty. The origins and consequences of these vapour pores are discussed.

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SO 3

Ceramide mediated silencing of the unfolded protein response in Saccharomyces cerevisiae

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Synthetic gene array analyses identify Tlg2 t-SNARE function as a key contributor to sec14-1ts yeast mutant vitality, and that Sec14 and Tlg2 both contribute to endosomal/TGN dynamics in yeast. Paradoxically, functional depletion of both Sec14 and Tlg2 manifests endoplasmic reticulum (ER) dysfunction through compromise of the unfolded protein response. Lipidomic, biochemical and genetic data connect deranged ceramide homeostasis to UPR failure in sec14-1ts tlg2 double mutants. Furthermore, the Sit4 ceramide-activated protein phosphatase (CAPP) is identified as the primary agent that links ceramide derangements with UPR failure. We propose that derangements in endosomal/TGN dynamics imposed by combinatorial Sec14/Tlg2 dysfunction disrupts sphingolipid homeostasis such that inappropriate turnover of complex sphingolipids occurs in the endosomal system. This circumstance activates CAPP dependent pathways for downregulation of UPR signaling in yeast. The collective results potentiate roles for endosomal/vacuolar membranes as hubs for biological regulation in eukaryotes.

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SO 4

Hyphenated techniques in clinical Phospholipidomics

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It is still a challenging task to analyze underivatised lipids and many different analytical techniques have been proposed to characterize pathophysiological deviations of the native lipid composition. Further more lipids are involved in cellular signaling and in cell death (apoptosis, necrosis). Eukaryotic cell membranes consist of different phospholipid classes with extremely varying concentrations. They consist of a polar head group (i.e. choline, serine, ethanolamine) esterified by a phosphate group to the sn-3 position of glycerol and varying saturated and unsaturated fatty acids at sn-1 and sn-2 position.