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).

Acknowledgment: Support from FCT (Portugal) is acknowledged for both grants and research projects funding.

References

- Castro, et al., 2007. *Biophys. J.* 93, 1639–1650.
de Almeida, et al., 2003. *Biophys. J.* 85, 2406–2416.
de Almeida, et al., 2005. *J. Mol. Biol.* 346, 1109–1120.
de Almeida, et al., 2007. *Biophys. J.* 93, 539–553.
Silva, et al., 2006. *Mol. Membr. Biol.* 23, 137–150.
Silva, et al., 2007. *Biophys. J.* 92, 502–516.

doi:10.1016/j.chemphyslip.2008.05.005

PL 3

The biology of metazoan-specific phosphatidylinositol transfer protein

Vytas A. Bankaitis

Department of Cell & Developmental Biology, Lineberger Comprehensive Cancer Center, School of Medicine, University of North Carolina, Chapel Hill 27599-7090, USA

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 PITP α function in the mouse, and the pathologies associated with functional ablation of this protein.

doi:10.1016/j.chemphyslip.2008.05.006

SO 1

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

W.K. den Otter¹, R. Notman², J. Anwar³, M.G. Noro⁴, W.J. Briels¹

¹ Computational Biophysics, University of Twente, Enschede, The Netherlands

² Molecular Biophysics, King's College London, London, UK

³ Computational Biophysics, Institute of Pharmaceutical Innovation, University of Bradford, UK

⁴ Physical and Chemical Insights Group, Unilever R&D Port Sunlight, UK

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 dimethylsulfoxide (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-

bic pores, i.e. the lipid tails are exposed at the membrane edge. Although hydrophobic pores are commonly envisaged to contain water, we find that nanometer-sized pores are actually empty. The origins and consequences of these vapour pores are discussed.

doi:10.1016/j.chemphyslip.2008.05.007

PL 4

Regulated assembly of proteins and lipids at the Golgi to generate membrane fission activity

Vivek Malhotra

CRG- Centre for Genomic Regulation, Barcelona, Spain

I will discuss our findings on the mechanism of transport carriers formation of the Golgi to cell surface pathway. This process is initiated by cargo, which through the involvement of GPCR, trimeric G-protein and Diacylglycerol (DAG); recruits protein kinase D (PKD) to the Golgi membranes. PKCeta activates PKD; the activated PKD then controls events leading to the fission [cutting] of cargo filled transport carriers. PKD has a number of substrates on the Golgi membranes, which interestingly are involved in regulating the Golgi specific levels of phosphoinositide (PI4P) and ceramide. I will discuss how these modified lipids are involved in membrane fission. New microscopic data will be discussed to highlight novel intermediates in the formation of Golgi to cell surface transport carriers. Our recent attempts to reconstitute this process in vitro to reveal the role of proteins in the generation of membrane destabilizing-modified lipids, to form transport carriers will be presented.

Additional reading

Bossard, C., Bresson, D., Polishchuk, R.S., Malhotra, V., 2007. Dimeric PKD regulates membrane fission to form transport carriers at the TGN. *J. Cell Biol.* 179, 1123–1131.

Bard, F., Malhotra, V., 2006. The formation of TGN to cell surface specific transport carriers. *Annu. Rev. Cell Dev. Biol.* 22, 439–455.

Bard, F., Casano, L., Mallabiarrena, A., Wallace, E., Dasgupta, R., Perrimon, N., Malhotra, V., 2006. Functional analysis of the drosophila genome reveals new components involved in protein secretion and Golgi organization. *Nature* 439, 604–607.

doi:10.1016/j.chemphyslip.2008.05.008

SO 2

Plasma membrane order in T cell signalling

Jelena Dinic, Jeremy Adler, Ingela Parmryd

Department of Cell Biology, The Wenner-Gren Institute, Stockholm University, Sweden

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 used to study membrane order. It undergoes a 50 nm emission shift depending on the environment—liquid ordered/solid or liquid disordered. T cell signalling can be initiated by stimulating the T cell receptor (TCR), crosslinking the lipid raft marker GM1, a sphingolipid, or glycosylphosphatidylinositol (GPI) anchored proteins. The aggregation of lipid raft components induces the same type of response in T cell as the binding of antigen to the TCR. By live imaging of Jurkat T cells at 37 °C under described crosslinking conditions, we followed the changes in membrane order linked with reorganization of plasma membrane upon T cell activation. Fluorescent images were analyzed for pixel intensity ratio between the

two channels representing Laurdan fluorescence in two different environments. ConA crosslinking, which causes aggregation of glycosylated membrane components, increases the overall membrane order of Jurkat T cells, leading to a speculation that it might be creating a solid gel phase. Binding of cholera toxin B (CT-B) which is pentavalent for GM1, anti-TfR (transferin receptor) or anti-CD59 does not seem to significantly influence overall membrane order of Jurkat T cells. Further analysis will compare the membrane order within patches with that of the bulk membrane in cells that have been patched.

doi:10.1016/j.chemphyslip.2008.05.009

SO 3

Ceramide mediated silencing of the unfolded protein response in *Saccharomyces cerevisiae*

Carl J. Mousley¹, Kimberly Tyeryar¹, Kristina E. Ile¹, Gabriel Schaaf¹, Renee Brost², Charles Boone², Xiuli Guan³, Markus R. Wenk³, Vytas A. Bankaitis¹

¹ University of North Carolina at Chapel Hill, USA

² C.H. Best Institute, University of Toronto, Canada

³ Department of Biochemistry and Biological Sciences, The National University of Singapore, Singapore

Synthetic gene array analyses identify Tlg2 t-SNARE function as a key contributor to *sec14-1^{ts}* 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 *sec14-1^{ts} 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.

doi:10.1016/j.chemphyslip.2008.05.010

SO 4

Hyphenated techniques in clinical Phospholipidomics

Jan Willmann^{1,2}, Dieter Leibfritz¹

¹ University of Bremen, Institute of Organic Chemistry, Leobener Str. NW 2C, 28359 Bremen, Germany

² Bruker Daltonik GmbH, Fahrenheitstraße 4, 28359 Bremen, Germany

It is still a challenging task to analyze underivatized 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.