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meeting point

Lipids as organizers of cell membranes

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The 105th Boehringer Ingelheim Fonds International Titisee Conference 'Lipids as Organizers of Cell Membranes' took place in March 2012, in Germany. Kai Simons and Gisou Van der Goot gathered cell biologists and biophysicists to discuss the interplay between lipids and proteins in biological membranes, with an emphasis on how technological advances could help fill the gap in our understanding of the lipid part of the membrane.

Lipid membranes are barriers that individualize cells and intracellular compartments. Whereas recombinant DNA techniques have allowed the rapid exploration of gene control and protein functions, the lipid world has remained partly unexplored, as the variety of lipid chemistry is not directly genetically encodable. The early model for lipid membranes proposed by Singer and Nicolson in 1972 [1] was based on the idea that membranes are essentially made by the spontaneous assembly of phospholipids in a two-dimensional fluid bilayer, in which membrane proteins are embedded and freely diffuse. As this structure is crucial for membrane functions, both the chemical nature of lipids and the physicochemical properties of the lipid bilayer have to be addressed. Among these properties, it is fluidity, diffusion and the lateral segregation of lipids and proteins into domains that have received the most focus, as they are essential to cell signalling, cell adhesion, membrane transport and cell polarization. However, it is difficult to genetically manipulate such systems, and the space and timescales at which these phenomena occur—less than a second at the nanometre range—initially constrained the advancement of this field. In response, scientists set about developing complex biophysical and chemical tools, including mass-spectrometry-based lipidomics, fluorescent correlation spectroscopy and structural and chemical biology, to name a few. Now, the fruits of those labours are ripening and the 105th International Titisee Conference 'Lipids as Organizers of Cell Membranes', exhibited many of the exciting advances in the field.

Control of membrane lipid composition

Proteins control lipid metabolism, but the enzymatic reactions that lead to the synthesis of a particular lipid are only the first of many steps needed to achieve homeostasis of the membrane bilayer. A lipid needs to be transported to its destination and its abundance to be controlled, at the level of both the cell and the organism.

Answering the question of how the abundance of a given lipid is regulated, Joost Holthuis (U. Osnabrück, Germany) presented work on a member of a family of sphingomyelin synthases that function as protein sensors for the sphingolipid precursor ceramide. Loss of sensor function causes the deregulation of ceramide levels and the activation of a mitochondrial pathway of apoptosis.

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Lipid accumulation in a membrane can, in principle, be regulated at the levels of synthesis and turnover but also by lipid transport. Lipid transport is a part of vesicular membrane transport, but is also performed by transporters capable of shuttling lipids across an aqueous phase. Contact sites between membranes might facilitate transport by reducing the distance. Mitochondria are prime examples of this latter type of transport, as they are separate from classical vesicular transport routes. Two talks, one by Will Prinz (The National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, USA) and another by Benoît Kornmann (ETH Zürich, Switzerland), showed how a protein complex that tethers the mitochondria to the endoplasmic reticulum is necessary for an appropriate lipid balance in the mitochondrial membranes of yeast. Pietro De Camilli (Yale U., USA) and Kai Simons (MPI of Molecular Cell Biology and Genetics, Germany) further explored the importance of the proper localization of lipids. De Camilli described how the proper localization of phosphoinositides, cholesterol and sphingolipids is crucial for the function of proteins interacting transiently with the membrane in endocytic vesicle budding and fission. Simons addressed how glycolipids partition between basolateral and apical membranes during induced cell polarization, and how this partitioning drives polarized non-conventional exocytosis events.

How are lipids that enter from the extracellular milieu processed and how do they reach their destinations? Elina Ikonen (U. Helsinki, Finland) addressed this question by following the cellular fate of low-density lipoprotein-derived cholesterol from endolysosomes to the plasma membrane.

The chemical nature of the lipids incorporated into a given membrane changes many properties of that membrane. One of them is thickness. Several talks addressed interesting phenomena that are influenced by bilayer thickness. Diego de Mendoza (Institute of Molecular and Cell Biology of Rosario, Argentina) described how a bacterial kinase/phosphatase uses membrane thickness as a proxy to probe for membrane fluidity. Membrane thickness also influences membrane transport. In the context of endoplasmic reticulum protein exit, the lab of Maya Schuldiner (Weizmann Institute, Israel) showed that a large class of different cargo is sorted to endoplasmic reticulum exit sites by a cargo receptor, Erv14, which recognizes its wide

substrate range by the length of their transmembrane domain (TMD). Erv14's cargo reside in the thick membranes of the late Golgi or plasma membrane, and therefore have long TMDs relative to the thin endoplasmic reticulum membrane. In mammalian cells, Gisou Van der Goot (EPFL, Switzerland), described the importance of palmitoylation for proteins with long TMDs to find their way out of the endoplasmic reticulum, and for endoplasmic reticulum-resident proteins to partition between the peripheral and perinuclear endoplasmic reticulum. How these TMDs are inserted in membranes by the translocon remains a mysterious process. Gunnar Von Heijne (Stockholm U., Sweden) described an elegant system to measure the interaction of TMDs with the translocon, by probing the forces imposed by TMD–translocon interactions on the translating ribosome.

Shaping of membrane bilayers

At the macroscopic level, the lipid bilayer is best described as a two-dimensional (2D) fluid, auto-sealable structure. This model implies that the bending, stretching, fusion and fission of lipid bilayers all require energy and force, which are transmitted to the membrane by proteins involved in membrane transport.

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Endocytosis requires the budding of vesicles by local curvature of the plasma membrane. Harvey McMahon (Cambridge U., UK) discussed how epsins deform the membrane on its cytosolic side. Ludger Johannes (Institut Curie, France) described how lectins induce local curvature of the plasma membrane from the extracellular side.

Membrane curvature is not only important for vesicle budding, but also for vesicle abscission. Aurélien Roux (U. Geneva, Switzerland) showed that dynamin does not complete vesicle abscission by constricting the membrane down to a point where the bilayer is disrupted, but instead by imposing a change in membrane curvature that renders the membrane spontaneously fusogenic. Fusogenicity is also required for multivesicular body formation. In this case, the endosomal sorting complexes required for transport (ESCRT) can bud and sever vesicles in a topological setup that is opposite to that of clathrin and dynamin, promoting the scission of membrane necks from the inside. Jim Hurley (National Institutes of Health, USA) presented structural data on how ESCRT proteins achieve such a feat.

Other areas of great membrane curvature are caveolae. Rob Parton (U. Queensland, Australia) presented an interesting new function for these domains: a reserve of membrane surface necessary to withstand cell enlargement consequent to muscular extension.

Diffusion of proteins and lipids

Proteins are not the only factors to influence membrane composition, shape and function. Lipids also play a central role in setting the fate and functions of the proteins they encounter within membranes.

One rising matter is how lipids and proteins diffuse together: can lipid diffusion be altered by the presence of proteins? Do the clustering and diffusion properties of membrane proteins change depending on the lipid environments with which they are associated? Hai-Tao He (Centre for Immunology of Marseille-Luminy, France) addressed these questions directly by measuring the diffusion of glycophosphatidylinositol (GPI)-anchored proteins, known to associate with detergent-resistant membranes, by fluorescence correlation spectroscopy (FCS) on the membrane of cells. He found that GPI-anchored proteins experience constrained lateral diffusion with dynamic partitioning into domains of less than 100 nm. Christian Eggeling (MPI for Biophysical Chemistry, Germany) presented results that lead him to conclude that certain lipids and proteins form transient complexes on molecular scales. He developed improved FCS measurement performed with stimulated emission depletion microscopy, which allows a unique reduction of the size of the observation area from 200 nm down to 30 nm in diameter. Both He and Eggeling found that diffusion could be altered strongly, by using drugs that interfere not only with the levels of cholesterol or sphingolipids, but also, interestingly, with the dynamics of the actin cortex. Furthermore, Eggeling indicated no correlation between the observed live-cell interactions and phase separation in model membranes. By using total internal reflection fluorescence, as well as fluorescence resonance energy transfer (FRET) imaging in conjunction with FCS, and predictions from a theoretical model, Satyajit Mayor (National Centre for Biological Sciences, India) came to the conclusion that membrane proteins and lipids can be actively and hierarchically clustered by the dynamics of short actin filaments at the cytosolic leaflet of the membrane. This mechanism suggests the active control of local membrane composition dictated by a dynamic cytoskeleton. Akihiro Kusumi (Kyoto U., Japan) showed that the effect of actin is strongly dependent on whether membrane proteins have a direct or passive interaction with the actin cortex. These four studies on protein diffusion indicate that the size and the lifetime of these membrane protein nanoclusters depend both on lipid segregation into domains, as well as on the dynamics of the actin cortex. The exact importance of each contribution seems variable.

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Kusumi also reported that dimeric proteins form more stable clusters, although these are still sensitive to lipid and actin drugs. This suggests that the clustering of membrane proteins could induce lipid domains. Sarah Veatch (U. Michigan, USA) used a reconstituted giant liposome system to show that clustering of proteins through adhesion of the liposome to a surface can stabilize extended domains of certain lipids even in a single phase membrane. Gerhard Schütz (Vienna U. of Technology, Austria) showed that cells adhering to a patterned surface with antibodies directed against GPI-anchored proteins show segregation of all GPI-anchored proteins, even of those not recognized by the antibodies. These presentations brought up the idea that the diffusion of membrane proteins was dependent on membrane factors—such as lipid composition and segregation—as well as on factors external to the membrane—such as actin dynamics and ligand binding.

Can lipid diffusion be modified by the presence of membrane proteins? Ilpo Vattulainen (U. Tampere, Finland) used numerical simulation to show that the

diffusion of lipids around transmembrane proteins can be severely reduced, even at a distance of a few nanometres. The dynamics of lipids could be impeded by direct hydrophobic interactions with the transmembrane core of the protein, or by kinetic trapping within 'pockets' between TMD segments. Tom Walz (Harvard U., USA) beautifully confirmed this prediction by showing that lipids directly in contact with the hydrophobic core of the protein adopt specific conformations stable enough to be observed by electron diffraction. Together with the role of the size of the hydrophobic helices discussed above, these results highlight the fact that TMDs do not float passively in the bilayer; they actually interfere strongly with the diffusion, local composition and physicochemical state of the lipids.

Influence of bilayers on signalling

What is the physiological role of such protein control on the bilayer state? Mark Davis (Stanford U., USA) and Michael Dustin (New York U., USA) showed that the same interplay between lipid phase separation, actin dynamics and protein clustering is crucial for signal transduction in the context of T-cell activation. Whilst interacting with antigen-presenting cells, T cells form an adhesive structure called the immunological synapse that concentrates the T-cell receptors (TCRs) in its centre, circled by adhesive molecules that stabilize the structure. T lymphocytes are crucially dependent on actin dynamics, protein–protein interactions and lipid phase segregation for the formation of the immunological synapse. Davis measured the K_{off} of the multiple histocompatibility complex (MHC)–TCR interaction with FRET in T cells interacting with supported bilayers containing MHC. By using various drugs, he showed that actin dynamics destabilizes the MHC–TCR interaction. Thus the formation of the cluster leading signalling was dependent on actin dynamics, lipid segregation and protein–protein interactions. By using T cells interacting with a MHC supported bilayer, Dustin studied the structure of the synaptic cleft using various microscopy techniques. He found that the maturation of the synapse was concomitant with the appearance of small vesicles within the cleft, the formation of which was ESCRT-III dependent. The vesicles remained linked to the supported bilayers after disruption of the immunological synapse, and if B cells were put in contact with these vesicles, they became activated. Transfer of these extracellular vesicles appears to be a new mode of communication across a synapse.

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Last but not least, microtubules were brought in to the picture by Gillian Griffiths (Cambridge U., UK), who showed that another immunological synapse, formed by natural killer cells with antigenic cells, relies on the presence of the centrosome, or microtubule-organizing centre, for orientation and docking to the antigenic cell. Her data described the remarkable similarities between the establishment of this immunological synapse and of the specialized membrane of the cilium, with centrosome docking leading to membrane specialization.

In a totally different context, John Hancock (Texas U., USA) showed that Ras signalling is strongly dependent on the ability of K-Ras to form nanoclusters. Surprisingly, as seen by electron microscopy, GTP-bound and GDP-bound Ras form different nanoclusters. Hancock showed that the precise gain of the Ras signal was strictly dependent on the formation of these.

The focus of the Titisee meeting was on lipid dynamics. Judging by the topics presented, it is clear that lipids are not mere solvents for membrane proteins, but that they play active roles in the organization, shaping and function of membranes. Whilst proteins synthesize, regulate and distribute lipids to the numerous membranes of cells, lipids in turn interact with proteins, determining their function, localization to organelles and sequestration into nanoclusters and rafts. The importance of the interplay between lipids and proteins is best seen in its effect on membrane function: any attempt to perturb actin, protein–lipid or protein–protein interaction leads to defects in signalling, sorting and budding of membrane components. In short, this interplay affects organelle homeostasis, vesicular transport and intra- and inter-cellular signalling, and is therefore central to cell physiology.

To better observe the complexity of membrane dynamics, the attendees of the Titisee meeting presented the use of a great variety of tools lying at the interface between physico-chemistry and biology. These tools convey the hope that lipid dynamics will soon reveal some of its secrets. In organizing this meeting, Gisou Van der Goot and Kai Simmons, as well as Boehringer Ingelheim Fonds, allowed us to explore new routes and discover new tools. On behalf of all participants, we thank them for putting together such an exiting meeting.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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