Lipid map of the mammalian cell

Gerrit van Meer and Anton I. P. M. de Kroon

Bijvoet Center and Institute of Biomembranes, Utrecht University, Padualaan 8, NL-3584 CH Utrecht, The Netherlands

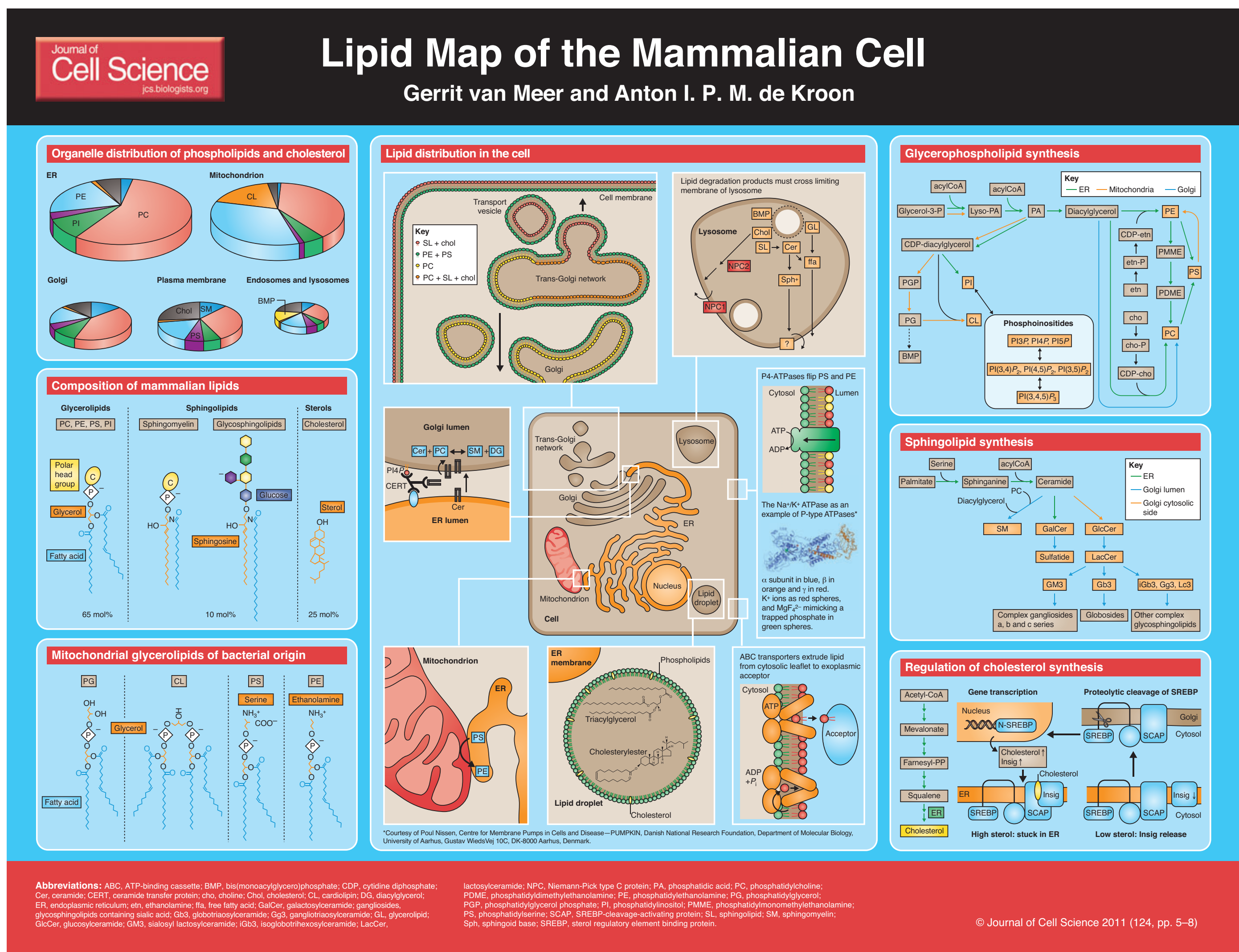
(g.vanmeer@uu.nl; a.i.p.m.dekroon@uu.nl)

Journal of Cell Science 124, 5-8
© 2011. Published by The Company of Biologists Ltd
doi:10.1242/jcs.071233

Technological developments, especially in mass spectrometry and bioinformatics, have revealed that living cells contain thousands rather than dozens of different lipids [for classification and nomenclature, see Fahy et al. (Fahy et al., 2009)]. Now, the resulting questions are what is the relevance of each of these unique molecules for the cell and how do cells use lipids for their vital functions? The answer requires an integrative approach – cellular lipidomics – which addresses first the distribution of all lipids between the various organelle membranes and then their local organization within each membrane. To understand lipid homeostasis and its dynamics, one has to study the localized metabolism of lipids, their transport within and between the various membranes, and the sensors and effectors that govern these processes. In terms of function, above all, we need to understand the physical behavior of complex lipid mixtures and their effect on local protein structure, organization and function. Finally, in the course of evolution, many lipids and lipid metabolites have acquired key functions in the signaling networks that wire the cell, by binding to cognate receptors and by recruiting proteins to specific membranes. The accompanying poster describes the lipid content of the various organelle membranes, illustrates lipid localization and dynamics in various subcellular locations, and explains the structure of lipids and their biosynthetic pathways. Below, we highlight additional issues that are important in lipid cell biology, and aim to provide a framework and a timely update for lipid systems biology.

Lipid self-organization and subcellular distribution

Bacteria, archaea and eukaryotes share glycerol as the backbone of most of their lipids. The typical bacterial phospholipids are phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and cardiolipin (CL), which are also found in eukaryotes. PG and CL are synthesized in and confined to mitochondria (see Poster). Mitochondria also harbor the (bacterial) enzyme PS-decarboxylase (PSD), which synthesizes half of the cellular PE. Phosphatidylcholine (PC) and phosphatidylinositol (PI) are the other major eukaryotic glycerophospholipids. Owing to its two fatty acyl chains and a large polar head, PC has a



(See poster insert)

cylindrical shape. Because the entropy is highest when the lipid tails are turned away from water and water molecules have maximum freedom (the 'hydrophobic effect'), PC molecules assemble into a bilayer. The typical PC carries one saturated and one unsaturated chain. It yields a fluid ('liquid crystalline') membrane with many characteristics of biomembranes. However, biomembranes typically contain five to ten major lipid classes (see Poster), which are needed for processes such as vesicle fusion and fission, membrane sorting and signal transduction.

Most PE molecules found in biological membranes are cone shaped and don't form lipid bilayers by themselves. The non-bilayer propensity of PE is essential for the functional embedding of membrane proteins and for processes such as membrane fusion and fission. Under conditions of charge neutralization (divalent cations or high salt), mitochondrial CL also acquires a preference for a non-bilayer configuration. Inactivation of CL synthase and mitochondrial PSD in yeast is synthetically lethal (Gohil et al., 2005), indicating a requirement for mitochondrial non-bilayer lipids. The non-bilayer propensity of PE and CL depends on the length and level of unsaturation of their acyl chains. The acyl chain composition is modulated by acyl chain exchange, which is catalyzed by remodeling enzymes. The new developments in mass spectrometry have enabled us to monitor lipid remodeling in living cells (de Kroon, 2007) and also allowed rapid progress in the area of ether lipids. Up to 50% of glycerolipids contain ether-linked chains, but their functions remain largely unknown (Lessig and Fuchs, 2009).

In addition to the glycerol-based phospholipids, eukaryotes invariably possess sphingolipids and sterols. Sphingolipids usually contain a long to very long saturated fatty acid (C16–C32) with an amide linkage to the sphingoid base (Poster). Variations such as C2-hydroxylation and C15-unsaturation are not uncommon. Sphingolipids generally adopt a solid gel phase, but are fluidized by sterols, which preferentially interact with them in the membrane. Sphingolipids and sterols are enriched in the plasma membrane and in endosomes. They render these membranes exceptionally sturdy. PC and PI are enriched in the endoplasmic reticulum (ER) [see Poster; diameters reflect contribution to total cellular lipid; calculated from Zambrano et al. (Zambrano et al., 1975) and Griffiths et al. (Griffiths et al., 1989)]. Similar to the bacterial membrane, the thin and flexible ER is involved in the insertion of membrane and secretory proteins. In addition to the differences between organelles, the two leaflets of (post-)Golgi membrane bilayers also have different lipid compositions (Bretscher, 1972; Verkleij et al., 1973; Simons and van Meer, 1988) (see Poster). The sphingolipids are synthesized on the lumenal surface of the Golgi membrane and are found on the outside of the plasma membrane, whereas the aminophospholipids PS and PE are actively concentrated in the cytosolic leaflet (see below). Because of the preferential interaction of cholesterol with sphingolipids, it should be enriched in the non-cytosolic leaflet of the membrane. However, experimental evidence instead suggests a very high ratio between cholesterol and phospholipids in the cytosolic leaflet (Mondal et al., 2009). This finding is presently difficult to interpret in physical terms.

Lipid transport

Flippases stabilize transbilayer lipid asymmetry

In pure lipid membranes, the polar head group of the regular phospholipids does not readily pass through the hydrophobic membrane interior. This is also true for both the erythrocyte membrane, for which the half-time of translocation across the bilayer for PC was found to be greater than 10 hours, and (post-)Golgi membranes in nucleated cells. By contrast, the various phospholipids move rapidly (in the order of seconds) across the ER membrane in an energy-independent process mediated by (so far) unknown proteins (Sanyal and Menon, 2009).

Lipid asymmetry across biomembranes is dynamic (Seigneuret and Devaux, 1984). Each (post-)Golgi membrane contains P4-ATPases, members of the cation-transporting P-type ATPase family. P4-ATPases translocate the aminophospholipids PS and PE towards the cytosolic leaflet (Tang et al., 1996) [see Poster, structure of a related pump (cf. Pedersen et al., 2007)], and are generally termed 'flippases'. The activation of so-called 'scramblase' activity allows lipid mixing between the leaflets and exposes PS on the cell surface (Bevers and Williamson, 2010). This occurs late in apoptosis, after which PS is recognized by a PS receptor (Wong et al., 2010) and the apoptotic cell undergoes phagocytosis. In addition, PS exposure on blood cells or platelets signals blood coagulation. Flippases maintain lipid asymmetry, but the net translocation of lipid mass from one bilayer leaflet to the other also leads to curvature of the membrane. This possibly drives the budding of transport vesicles in post-Golgi vesicle trafficking (Leventis and Grinstein, 2010). Lipids without sizeable head groups, such as cholesterol, diacylglycerol (DG), ceramide and fatty acids (ffa) in their protonated form, readily translocate spontaneously. Nevertheless, the export of lipoprotein-derived cholesterol from lysosomes requires the Niemann-Pick disease type C protein 1 (NPC1) in the lysosomal membrane. Instead of acting as a flippase, NPC1 probably inserts low-density lipoprotein (LDL)-derived cholesterol from the lumen into the surrounding membrane across the glycocalyx, the protective layer of glycans on the inner surface of the lysosomal membrane (see Poster) (Kolter and Sandhoff, 2009). This is then followed by its spontaneous transmembrane translocation. NPC1 might also translocate lumenal sphingoid bases, which are positively charged at lysosomal pH, across the lysosomal membrane (Lloyd-Evans et al., 2008).

A number of ATP-binding cassette (ABC) transporters move lipids away from the cytosol (see Poster). However, they don't deposit the lipids into the non-cytosolic membrane leaflet using a reversed flippase ('floppase') mechanism. In most cases, ABC transporters extrude the lipid substrate onto acceptors outside the membrane (van Meer et al., 2006). Glucosylceramide, the only glycosphingolipid synthesized on the cytosolic surface of the Golgi, requires a floppase to be able to reach the Golgi lumen (D'Angelo et al., 2007), where it is converted into a higher glycosphingolipid (see Poster). We observed no direct translocation across the Golgi membrane. Instead, glucosylceramide reached the Golgi lumen through the ER, mediated by the glucosylceramide-binding protein FAPP2 (Halter et al., 2007). Interestingly, the related galactosylceramide is synthesized in the ER (Sprong et al., 1998). Both glycosphingolipids can also reach the cytosolic side of the plasma membrane, across which they can be translocated (Halter et al., 2007), possibly by ABCA12, a putative glucosylceramide exporter in the skin (Jiang et al., 2009).

Lipid sorting in vesicular pathways by lateral segregation in domains

How do cells generate and maintain the differences in lipid composition between their organelles? Lipids rapidly diffuse laterally in the membrane plane, typically 1 μm^2 per second. Therefore, the budding and fusion of membrane vesicles with a typical surface area of 0.02 μm^2 (which occurs in the order of seconds) should mix the lipids of the organelles along the vesicular pathways. However, physical differences between the glycerolipids and sphingolipids make them segregate into two distinct fluid phases in the presence of 5–50 mol% cholesterol, its biological concentration range (Marsh, 2009). In (post-)Golgi membranes, domains of different lipid composition are targeted into separate carrier

Journal of Cell Science

Journal of Cell Science

vesicles with unique protein labels to address them to, for example, the ER and plasma membrane (see Poster). This segregation of lipids and proteins forms the basic sorting mechanism by which cells maintain the unique lipid composition of their membranes (Simons and van Meer, 1988; van Meer et al., 2008). Sophisticated cell fractionation and lipidome analysis has shown that retrograde Golgi-derived vesicles, identified by their COPI coat, are enriched in ER lipids (Brügger et al., 2000). Anterograde vesicles from the trans-Golgi network (TGN) are enriched in sphingolipids and sterols (Klemm et al., 2009). When, during evolution, cells acquired the ability to synthesize sphingolipids and sterols, they also developed their endomembrane system (Freilich et al., 2008; Desmond and Gribaldo, 2009). This suggests that lipid-based sorting is a fundamental eukaryotic property. Many molecular details remain unclear, for example, how membrane proteins concentrate in a given domain. On the plasma membrane, sphingolipid–cholesterol nanodomains with their specific proteins, termed lipid rafts, supposedly function as transient signaling platforms. Their size and life-time are subject of ongoing investigations.

Transfer proteins and contact sites guide lipid monomers through the cytosol

Various families of cytosolic proteins can bind and solubilize lipid monomers. Some of these belong to the nuclear receptor family of transcription factors. Others transfer (phospho)lipids between membranes. Ceramide transfer protein (CERT) is required for transporting newly synthesized ceramide from the ER to the trans-Golgi, where it is metabolized to sphingomyelin (SM) (see Poster). Golgi synthesis of glucosylceramide was shown to be CERT independent (Hanada et al., 2003) (cf. Halter et al., 2007). CERT binds to a protein on the ER and to the signaling lipid phosphatidylinositol 4-phosphate (PI4*P*) on the trans-Golgi, probably at contact sites between these organelles. The transport activity of CERT, and thereby SM synthesis, is regulated by the concentration of PI4*P* (see below) and the phosphorylation state of CERT (Hanada et al., 2003). CERT-related proteins possessing the same ER and Golgi binding sites bind oxysterols, whereas another type of oxysterol-binding protein was found to be located at the ER–endosome and Golgi–endosome interfaces (Raychaudhuri and Prinz, 2010). Contact sites have been found between the ER and mitochondria, Golgi, the plasma membrane and lipid droplets. Their molecular structure and their function in transporting lipids, proteins and ions are the subject of intense research. The first protein complexes at ER–mitochondria contact sites have been identified (de Brito and Scorrano, 2008; Kornmann et al., 2009). They indeed appear to be involved in the transport of PS from the ER to mitochondria and of PE resulting from PS decarboxylation back to the ER (see Poster). The finding that a protein of the mitochondrial protein import machinery affects the mitochondrial CL content (Kutik et al., 2008) will further help to resolve the molecular mechanisms that regulate mitochondrial lipid composition. The relationship between ER and lipid droplets, especially the role of the ER in droplet biogenesis, and the organization of the enzymes of lipid metabolism at the droplet surface (Poster) are not yet understood (Robenek et al., 2009).

Lipids as primary and secondary messengers: topology

During evolution, cells started to use specific lipids at specific locations for signaling purposes. First of all, the wide variety of glycosphingolipid structures on the cell surface, now evident from detailed mass spectrometric profiling (Li et al., 2010), impose specificity on interactions with glycans or lectins on other cells or in the extracellular matrix. They can also regulate the activation of the insulin and epidermal growth factor receptors (Bremer et al., 1986) and of integrins by modulating their organization in lipid rafts (Pike et al., 2005; Regina Todeschini and Hakomori, 2008). Various glycosphingolipids are targets of toxins, viruses, bacteria and parasites. Second, a dedicated complex regulatory system of lipid kinases and phosphatases provides individual organelles with unique derivatives of PI that are phosphorylated at one, two or three positions of the inositol ring: the phosphoinositides (see Poster). Examples are PI4*P* in the Golgi, phosphatidylinositol 3-phosphate (PI3*P*) in endosomes and phosphatidylinositol (4,5)-bisphosphate [PI(4,5)P_2] in plasma membranes (Di Paolo and De Camilli, 2006). The phosphoinositides on the cytosolic surface recruit organelle-specific effector proteins in vesicle trafficking and also in signal transduction. Inside the nuclear matrix, phosphoinositides are embedded in protein signaling complexes (Barlow et al., 2009).

The secondary messengers ceramide and DG, when produced by phospholipases at the plasma membrane, recruit cytosolic kinases and phosphatases. Ceramide and DG levels regulate apoptosis (Bartke and Hannun, 2009) and Golgi transport, respectively. Phospholipases A_2, C and D, which produce lysophospholipids plus fatty acids, DG and phosphatidic acid (PA), respectively, are required for budding and fusion at the Golgi (Asp et al., 2009; Judson and Brown, 2009; Riebeling et al., 2009; San Pietro et al., 2009) and probably other organelles. A number of more water-soluble lipids have been found to bind to cognate receptors; for instance, sphingosine-1-phosphate (S1P) and probably lysophosphatidic acid (LPA) are secreted by cells via ABC transporters (Takabe et al., 2010) and subsequently activate S1P and LPA receptors on the cell surface. They are involved in multiple aspects of cell proliferation and differentiation (Fyrst and Saba, 2010; Tigyi, 2010). Arachidonic acid, its derivatives and oxysterols are freely mobile in the cytosol and activate nuclear receptors that affect transcription. Finally, cells also employ sensor and effector systems to regulate the lipid composition of their membranes. One example is the sterol regulatory element binding protein (SREBP) system. When the cholesterol concentration in the ER gets below 5 mol% (Radhakrishnan et al., 2008), the sterol-sensing protein Insig is released from a complex with SREBP and SREBP-cleavage-activating protein (SCAP). This allows transport of SREBP–SCAP to the Golgi. There, the N-terminal cytosolic tail of SREBP is cleaved, yielding a transcription factor that regulates the expression of enzymes involved in cholesterol (i.e. SREBP-2) and fatty acid (i.e. SREBP-1) metabolism (Brown and Goldstein, 2009) (see Poster). Moreover, an ER member of the SM synthase family regulates the cellular ceramide concentration (and thereby apoptosis) and is a candidate ceramide sensor (Vacaru et al., 2009). Because oxysterols are relatively water soluble, they readily transfer between membranes. Therefore, oxysterol-binding proteins might function as sensors rather than transporters (Raychaudhuri and Prinz, 2010). A role as sensors in regulating vesicular transport has been proposed for PI-transfer proteins in yeast (Mousley et al., 2007).

Perspectives

One enigma in lipid biology is the dynamic organization of cholesterol. Although it can spontaneously move across and between membranes, many proteins have been found to stimulate its movement. Its high affinity for sphingolipids contrasts with the experimental results regarding its transbilayer organization. It also remains unclear how cells move domains of sphingolipids and cholesterol, which have an increased resistance against bending, into highly curved budding Golgi vesicles. Moreover, biophysicists do not see membrane proteins move into such 'ordered domains' in artificial reconstituted membranes, whereas they do in the cell. The molecular mechanism of flippases and their lipid specificity are not yet understood

nor is intermembrane lipid transport through membrane contact sites. The biogenesis of lipid droplets and their relationship with the ER constitutes another unresolved issue. Finally, we lack a basic understanding of how and why our cells synthesize the multitude of lipid species that we now observe with our sharpened analytical tools. We don't understand their impact on membrane structure and function, and we probably miss half of the functions that are exerted by lipids in signal transduction and homeostasis because they occur in minor amounts.

Many enzymes are involved in the synthesis, remodeling and conversion of cellular lipids, and their intermembrane and intramembrane transport. It is a challenge to unravel lipid homeostasis at the systems level; stable isotope labeling and mass spectrometry might allow us to do just that. A bigger challenge is to find out how lipid homeostasis ties in with all other protein-based systems in the cell to regulate cell physiology at large. Mapping the lipids is only a start!

References

Asp, L., Kartberg, F., Fernandez-Rodriguez, J., Smedh, M., Elsner, M., Laporte, F., Barcena, M., Jansen, K. A., Valentijn, J. A., Koster, A. J. et al. (2009). Early stages of Golgi vesicle and tubule formation require diacylglycerol. *Mol. Biol. Cell* **20**, 780-790.

Barlow, C. A., Laishram, R. S. and Anderson, R. A. (2009). Nuclear phosphoinositides: a signaling enigma wrapped in a compartmental conundrum. *Trends Cell Biol*. **20**, 25-35.

Bartke, N. and Hannun, Y. A. (2009). Bioactive sphingolipids: metabolism and function. *J. Lipid Res.* **50**, S91-S96.

Bevers, E. M. and Williamson, P. L. (2010). Phospholipid scramblase: an update. *FEBS Lett.* **584**, 2724-2730.

Bremer, E. G., Schlessinger, J. and Hakomori, S.-i. (1986). Ganglioside-mediated modulation of cell growth. Specific effects of GM3 on tyrosine phosphorylation of the epidermal growth factor receptor. *J. Biol. Chem.* **261**, 2434-2440.

Bretscher, M. S. (1972). Phosphatidyl-ethanolamine: differential labelling in intact cells and cell ghosts of human erythrocytes by a membrane-impermeable reagent. *J. Mol. Biol.* **71**, 523-528.

Brown, M. S. and Goldstein, J. L. (2009). Cholesterol feedback: from Schoenheimer's bottle to Scap's MELADL. *J. Lipid Res.* **50**, S15-S27.

Brügger, B., Sandhoff, R., Wegehingel, S., Gorgas, K., Malsam, J., Helms, J. B., Lehmann, W. D., Nickel, W. and Wieland, F. T. (2000). Evidence for segregation of sphingomyelin and cholesterol during formation of COPI-coated vesicles. *J. Cell Biol.* **151**, 507-517.

D'Angelo, G., Polishchuk, E., Di Tullio, G., Santoro, M., Di Campli, A., Godi, A., West, G., Bielawski, J., Chuang, C. C., van der Spoel, A. C. et al. (2007). Glycosphingolipid synthesis requires FAPP2 transfer of glucosylceramide. *Nature* **449**, 62-67.

de Brito, O. M. and Scorrano, L. (2008). Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature* **456**, 605-610.

de Kroon, A. I. (2007). Metabolism of phosphatidylcholine and its implications for lipid acyl chain composition in *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta* **1771**, 343-352.

Desmond, E. and Gribaldo, S. (2009). Phylogenomics of sterol synthesis: insights into the origin, evolution and diversity of a key eukaryotic feature. *Genome Biol. Evol.* **2009**, 364-381.

Di Paolo, G. and De Camilli, P. (2006). Phosphoinositides in cell regulation and membrane dynamics. *Nature* **443**, 651-657.

Fahy, E., Subramaniam, S., Murphy, R. C., Nishijima, M., Raetz, C. R., Shimizu, T., Spener, F., van Meer, G., Wakelam, M. J. and Dennis, E. A. (2009). Update of the LIPID MAPS comprehensive classification system for lipids. *J. Lipid Res.* **50**, S9-S14.

Freilich, S., Goldovsky, L., Ouzounis, C. A. and Thornton, J. M. (2008). Metabolic innovations towards the human lineage. *BMC Evol. Biol.* **8**, 247.

Fyrst, H. and Saba, J. D. (2010). An update on sphingosine-1-phosphate and other sphingolipid mediators. *Nat. Chem. Biol.* **6**, 489-497.

Gohil, V. M., Thompson, M. N. and Greenberg, M. L. (2005). Synthetic lethal interaction of the mitochondrial phosphatidylethanolamine and cardiolipin biosynthetic pathways in Saccharomyces cerevisiae. *J. Biol. Chem.* **280**, 35410-35416.

Griffiths, G., Back, R. and Marsh, M. (1989). A quantitative analysis of the endocytic pathway in baby hamster kidney cells. *J. Cell Biol.* **109**, 2703-2720.

Halter, D., Neumann, S., van Dijk, S. M., Wolthoorn, J., de Mazière, A. M., Vieira, O. V., Mattjus, P., Klumperman, J., van Meer, G. and Sprong, H. (2007). Pre- and post-Golgi translocation of glucosylceramide in glycosphingolipid synthesis. *J. Cell Biol.* **179**, 101-115.

Hanada, K., Kumagai, K., Yasuda, S., Miura, Y., Kawano, M., Fukasawa, M. and Nishijima, M. (2003). Molecular machinery for non-vesicular trafficking of ceramide. *Nature* **426**, 803-809.

Jiang, Y. J., Uchida, Y., Lu, B., Kim, P., Mao, C., Akiyama, M., Elias, P. M., Holleran, W. M., Grunfeld, C. and Feingold, K. R. (2009). Ceramide stimulates ABCA12 expression via peroxisome proliferator-activated receptor δ in human keratinocytes. *J. Biol. Chem.* **284**, 18942-18952.

Judson, B. L. and Brown, W. J. (2009). Assembly of an intact Golgi complex requires phospholipase A2 (PLA2) activity, membrane tubules, and dynein-mediated microtubule transport. *Biochem. Biophys. Res. Commun.* **389**, 473-477.

Klemm, R. W., Ejsing, C. S., Surma, M. A., Kaiser, H. J., Gerl, M. J., Sampaio, J. L., de Robillard, Q., Ferguson, C., Proszynski, T. J., Shevchenko, A. et al. (2009). Segregation of sphingolipids and sterols during formation of secretory vesicles at the trans-Golgi network. *J. Cell Biol.* **185**, 601-612.

Kolter, T. and Sandhoff, K. (2009). Lysosomal degradation of membrane lipids. *FEBS Lett*. **584**, 1700-1712.

Kornmann, B., Currie, E., Collins, S. R., Schuldiner, M., Nunnari, J., Weissman, J. S. and Walter, P. (2009). An ER-mitochondria tethering complex revealed by a synthetic biology screen. *Science* **325**, 477-481.

Kutik, S., Rissler, M., Guan, X. L., Guiard, B., Shui, G., Gebert, N., Heacock, P. N., Rehling, P., Dowhan, W., Wenk, M. R. et al. (2008). The translocator maintenance protein Tam41 is required for mitochondrial cardiolipin synthesis. *J. Cell Biol.* **183**, 1213-1221.

Lessig, J. and Fuchs, B. (2009). Plasmalogens in biological systems: their role in oxidative processes in biological membranes, their contribution to pathological processes and aging and plasmalogen analysis. *Curr. Med. Chem.* **16**, 2021-2041.

Leventis, P. A. and Grinstein, S. (2010). The distribution and function of phosphatidylserine in cellular membranes. *Annu. Rev. Biophys.* **39**, 407-427.

Li, Y., Arigi, E., Eichert, H. and Levery, S. B. (2010). Mass spectrometry of fluorocarbon-labeled glycosphingolipids. *J. Mass Spectrom.* **45**, 504-519.

Lloyd-Evans, E., Morgan, A. J., He, X., Smith, D. A., Elliot-Smith, E., Sillence, D. J., Churchill, G. C., Schuchman, E. H., Galione, A. and Platt, F. M. (2008). Niemann-Pick disease type C1 is a sphingosine storage disease that causes deregulation of lysosomal calcium. *Nat. Med.* **14**, 1247-1255.

Marsh, D. (2009). Cholesterol-induced fluid membrane domains: a compendium of lipid-raft ternary phase diagrams. *Biochim. Biophys. Acta* **1788**, 2114-2123.

Mondal, M., Mesmin, B., Mukherjee, S. and Maxfield, F. R. (2009). Sterols are mainly in the cytoplasmic leaflet of the plasma membrane and the endocytic recycling compartment in CHO cells. *Mol. Biol. Cell* **20**, 581-588.

Mousley, C. J., Tyeryar, K. R., Vincent-Pope, P. and Bankaitis, V. A. (2007). The Sec14-superfamily and the regulatory interface between phospholipid metabolism and membrane trafficking. *Biochim. Biophys. Acta* **1771**, 727-736.

Pedersen, B. P., Buch-Pedersen, M. J., Morth, J. P., Palmgren, M. G. and Nissen, P. (2007). Crystal structure of the plasma membrane proton pump. *Nature* **450**, 1111-1114.

Pike, L. J., Han, X. and Gross, R. W. (2005). Epidermal growth factor receptors are localized to lipid rafts that contain a balance of inner and outer leaflet lipids: a shotgun lipidomics study. *J. Biol. Chem.* **280**, 26796-26804.

Radhakrishnan, A., Goldstein, J. L., McDonald, J. G. and Brown, M. S. (2008). Switch-like control of SREBP-2 transport triggered by small changes in ER cholesterol: a delicate balance. *Cell Metab.* **8**, 512-521.

Raychaudhuri, S. and Prinz, W. A. (2010). The diverse functions of oxysterol-binding proteins. *Annu. Rev. Cell Dev. Biol.* **26**, 157-177.

Regina Todeschini, A. and Hakomori, S. I. (2008). Functional role of glycosphingolipids and gangliosides in control of cell adhesion, motility, and growth, through glycosynaptic microdomains. *Biochim. Biophys. Acta* **1780**, 421-433.

Riebeling, C., Morris, A. J. and Shields, D. (2009). Phospholipase D in the Golgi apparatus. *Biochim. Biophys. Acta* **1791**, 876-880.

Robenek, H., Buers, I., Hofnagel, O., Robenek, M. J., Troyer, D. and Severs, N. J. (2009). Compartmentalization of proteins in lipid droplet biogenesis. *Biochim. Biophys. Acta* **1791**, 408-418.

San Pietro, E., Capestrano, M., Polishchuk, E. V., DiPentima, A., Trucco, A., Zizza, P., Mariggio, S., Pulvirenti, T., Sallese, M., Tete, S. et al. (2009). Group IV phospholipase $A_2\alpha$ controls the formation of inter-cisternal continuities involved in intra-Golgi transport. *PLoS Biol.* **7**, e1000194.

Sanyal, S. and Menon, A. K. (2009). Flipping lipids: why an' what's the reason for? *ACS Chem. Biol.* **4**, 895-909.

Seigneuret, M. and Devaux, P. F. (1984). ATP-dependent asymmetric distribution of spin-labeled phospholipids in the erythrocyte membrane: relation to shape changes. *Proc. Natl. Acad. Sci. USA* **81**, 3751-3755.

Simons, K. and van Meer, G. (1988). Lipid sorting in epithelial cells. *Biochemistry* **27**, 6197-6202.

Sprong, H., Kruithof, B., Leijendekker, R., Slot, J. W., van Meer, G. and van der Sluijs, P. (1998). UDP-galactose:ceramide galactosyltransferase is a class I integral membrane protein of the endoplasmic reticulum. *J. Biol. Chem.* **273**, 25880-25888.

Takabe, K., Kim, R. H., Allegood, J. C., Mitra, P., Ramachandran, S., Nagahashi, M., Harikumar, K. B., Hait, N. C., Milstien, S. and Spiegel, S. (2010). Estradiol induces export of sphingosine 1-phosphate from breast cancer cells via ABCC1 and ABCG2. *J. Biol. Chem.* **285**, 10477-10486.

Tang, X., Halleck, M. S., Schlegel, R. A. and Williamson, P. (1996). A subfamily of P-type ATPases with aminophospholipid transporting activity. *Science* **272**, 1495-1497.

Tigyi, G. (2010). Aiming drug discovery at lysophosphatidic acid targets. *Br. J. Pharmacol.* **161**, 241-270.

Vacaru, A. M., Tafesse, F. G., Ternes, P., Kondylis, V., Hermansson, M., Brouwers, J. F., Somerharju, P., Rabouille, C. and Holthuis, J. C. (2009). Sphingomyelin synthase-related protein SMSr controls ceramide homeostasis in the ER. *J. Cell Biol.* **185**, 1013-1027.

van Meer, G., Halter, D., Sprong, H., Somerharju, P. and Egmond, M. R. (2006). ABC lipid transporters: extruders, flippases, or flopless activators? *FEBS Lett.* **580**, 1171-1177.

van Meer, G., Voelker, D. R. and Feigenson, G. W. (2008). Membrane lipids: where they are and how they behave. *Nat. Rev. Mol. Cell Biol.* **9**, 112-124.

Verkleij, A. J., Zwaal, R. F. A., Roelofsen, B., Comfurius, P., Kastelijn, D. and van Deenen, L. L. M. (1973). The asymmetric distribution of phospholipids in the human red cell membrane. A combined study using phospholipases and freeze-etch electron microscopy. *Biochim. Biophys. Acta* **323**, 178-193.

Wong, K., Valdez, P. A., Tan, C., Yeh, S., Hongo, J. A. and Ouyang, W. (2010). Phosphatidylserine receptor Tim-4 is essential for the maintenance of the homeostatic state of resident peritoneal macrophages. *Proc. Natl. Acad. Sci. USA* **107**, 8712-8717.

Zambrano, F., Fleischer, S. and Fleischer, B. (1975). Lipid composition of the Golgi apparatus of rat kidney and liver in comparison with other subcellular organelles. *Biochim. Biophys. Acta* **380**, 357-369.

Cell Science at a Glance on the Web
Electronic copies of the poster insert are available in the online version of this article at jcs.biologists.org. The JPEG images can be downloaded for printing or used as slides.