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Cellular organelles: how lipids get there, and back

Gerrit van Meer

*28th Steenbock Symposium, Intracellular Protein and Lipid Traffic; Madison, WI, USA; 12–14 August 2000. Organized by S.Y. Bednarek and A.K. Menon.

During the opening reception of the 28th Steenbock Symposium*, several groups of happy scientists could be observed hugging, kissing and shaking hands – all dependent on gender and nationality. Others clustered around food and drinks. These preferential interactions and lateral segregations were to remain a central theme in the lipid half of this meeting on 'Intracellular Protein and Lipid Traffic'. The other talks, not covered here, presented new developments in the various areas of protein translocation and traffic and in the budding, transport, docking and fusion of vesicles and organelles.

Translocators

It has long been known that the various organelles contain unique sets of lipids. Most notably, glycosphingolipids, sphingomyelin (SM), disaturated phospholipids and cholesterol are tenfold enriched in the plasma membrane as compared with the endoplasmic reticulum (ER)[1]. The ER membrane bilayer is symmetrical owing to the presence of a non-energy-requiring, ultrarapid, translocase (A. Herrmann, Berlin, Germany) for which first purification steps were presented[2]. The ER membrane has often been compared to bacterial inner membranes. Interestingly, in pure bacterial lipids, the presence of certain, but not other, simple peptides was sufficient to induce transbilayer diffusion of lipid probes (M. Kol, Utrecht, The Netherlands). In contrast to the ER, the plasma membrane is asymmetric. Its cytosolic leaflet is enriched in aminophospholipids by the 'aminophospholipid translocase' (Fig. 1). Its yeast homologue Drs2p might actually be a Golgi translocase (Z. Hua, Nashville, USA). New players in the field are the ABC transporters ABCB1 (the multidrug transporter hitherto known as MDR1 P-glycoprotein), which has now been demonstrated to be responsible for translocation of natural glucosylceramide from the cytosolic leaflet and into the exoplasmic leaflet of the plasma membrane of fibroblasts (G. van Meer, Amsterdam, The Netherlands), and ABCA1, the Tangier disease protein ABC1, which is essential for transport of cholesterol to extracellular high-density lipoprotein. In contrast to popular belief, transverse diffusion of cholesterol across the plasma membrane is slow[3] and might be speeded up by ABCA1 (A. Attie, pers. commun.). It is probable that ABCA1, ABCB1 and ABCB4 [the MDR3/mdr2 'phosphatidylcholine (PtdCho) translocator'] are actual translocators as they and other members of the family have been shown to pump amphipathic drugs, fatty acyl-coenzyme A molecules, organic anions and bile acids across membranes, possibly through a flippase mechanism[4]. Nevertheless, their activity and substrate specificity can only be assessed after functional reconstitution into pure lipid membranes. This is also true for the aminophospholipid translocase, especially because, in addition to the aminophospholipids phosphatidylserine (PtdSer) and phosphatidylethanolamine (PtdEtn), an inward translocator activity for (analogues of) PtdCho was reported here by several groups (A. Herrmann; G. van Meer; W. Nichols, Atlanta, USA).

Domains

Virtually all sphingolipids are synthesized on the lumenal aspect of Golgi membranes. Their enrichment on the cell surface has been explained by lateral segregation from phospholipids in the Golgi membrane and exclusion from retrograde transport vesicles[5]. COPI-coated vesicles originating from the Golgi contain less sphingomyelin and cholesterol (F. Wieland, Heidelberg, Germany)[6]. Assuming that these vesicles are responsible for retrograde transport, this supports the model. Anterograde concentration of cholesterol might be due to its preferential interaction with sphingolipids. Interestingly, the newly reported tight connection between the *trans* cisternae of the Golgi with ER[7] might allow

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cholesterol to pass from the ER directly into the *trans* Golgi, where it might induce or stabilize lateral phase segregation. Also, ceramide destined for sphingomyelin synthesis might enter the Golgi by this direct pathway as its route is different from the ER–Golgi vesicular pathway[8]. Preferential interactions of lipid anchors of proteins apparently drive their partitioning into sphingolipid/cholesterol domains. The fact that some of these anchors – the myristoyl and palmitoyl chains – are present on the cytosolic side of the membrane suggests that the lumenal sphingolipid domains might be accompanied by a domain of saturated phospholipids on the cytosolic side. In addition, the predicted increase in membrane thickness in the domains[9] is probably responsible for incorporation of specific integral plasma membrane proteins, which generally possess longer hydrophobic transmembrane domains[10].

Exocytosis

From using the decarboxylation of ER-derived PtdSer to PtdEtn in the Golgi/vacuole as an assay for PtdSer transport in yeast, two interesting proteins have been found so far – a phosphatidylinositol (PI) 4-kinase and a protein with homology to Sec14p, the yeast PI transfer protein. The new protein has high PI transfer activity[11] and might, like Sec14p, exert a regulatory function in its membrane-bound state. A screen for proteins required for retrograde lipid traffic to the ER using the conversion of new PtdEtn to PtdCho, which occurs in the ER, yielded a function for the Vam6p–Vam2p complex (D. Voelker, Denver, USA), hitherto implied in vacuolar morphogenesis. Cell-fractionation studies suggest that PtdSer and ergosterol follow the protein secretory route to the yeast plasma membrane, whereas PtdEtn and PI are transferred by other mechanisms (G. Daum, Graz, Austria). However, in other fractionation studies, ergosterol transport was found not to be influenced by the *sec18* mutation, which affects vesicular transport (C. Simonot, S. Bednarek, A. Menon, Madison, USA; cf. Ref. 12). PtdSer and PtdEtn transport into and out of the mitochondria probably occurs via ER–mitochondria contact sites[13].

Endocytosis

Lipid analogues have been used as bulk-flow markers for the endocytic recycling pathway. However, these calculations will have to be re-evaluated as it has now been found that lipid analogues with different physical characteristics partition into the various branches of the endocytic pathway to different extents (F. Maxfield, New York, USA). After insertion into the outer leaflet of the plasma membrane bilayer, sphingolipid analogues (bodipy-SM and bodipy-LacCer) that exhibit concentration-dependent fluorescence properties were sorted into different endosome populations during the first seconds of internalization. The LacCer analogue was endocytosed primarily through caveolae and was found to be transported to the Golgi via a nocodazole- and wortmannin-sensitive, Rab7-dependent pathway. This Golgi targeting was diverted to lysosomes in storage disease cells where cholesterol homeostasis is perturbed (R. Pagano, Rochester, USA)[14]. Natural lipids might be sorted by lateral segregation in the endocytic pathway as well. Interestingly, it was found that plasma membrane components can organize themselves in at least three different domains and that 80% of the plasma membrane lipids are in an ordered state as defined by resistance to detergent extraction (F. Maxfield). The idea of small ordered domains in a sea of fluid lipids seems to be a misconception.

Protein sorting

Lipids follow all vesicular pathways and they are involved in protein sorting. Unexpectedly, glycosphingolipids appear to be required for protein traffic from the Golgi to the melanosome via a 'non-raft' mechanism (G. van Meer; P. van der Sluijs, Utrecht, The Netherlands). Glucosylceramide, the only glycosphingolipid synthesized on the cytosolic surface of the Golgi, is possibly directly involved in coat recruitment. New synthesis of sphingoid bases is also required for transport of glycosylphosphatidylinositol (GPI)-linked proteins from the ER to the Golgi apparatus in yeast. The GPI-anchored proteins seem to exit the ER in vesicles that are distinct from those carrying other plasma membrane proteins (H. Riezman, Basel, Switzerland). In addition, specific lipids are produced transiently at sites of membrane budding and docking, where they are probably involved in recognition and fusion events by either changing the local physical properties of the membrane or by recruiting the necessary proteins. Phosphorylated PIs act in multiple vesicular pathways in yeast. A novel protein Vac14p regulates the activity of Fab1p, a kinase producing the unusual phospholipid phosphatidylinositol (3,5)-bisphosphate and is involved in vacuolar membrane traffic (C. Bonangelino, J. Urbanowski, J. Duex, L. Weisman and R. Piper, Iowa City, USA). Enigmatic lipid species in terms of cellular function are the phospholipids in which the hydrocarbon chain is connected to the glycerol C1 position by an ether, rather than an ester bond. Vesicular transport of cholesterol from plasma membrane and lysosome to the ER and fluid-phase endocytosis were grossly affected in mammalian cell mutants unable to synthesize such plasmalogens, whereas the secretory pathway was normal (L. Liscum and R. Zoeller, Boston, USA).

Lipids rapidly diffuse in the plane of the membrane, some flip across membranes and some cross the cytosol as monomers. The cell clearly succeeds in neutralizing the disruptive effects of

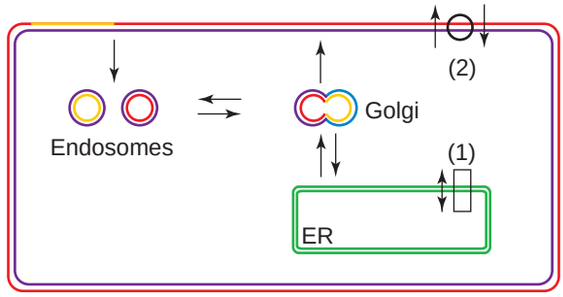

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FIGURE 1

Simple scheme of vesicular lipid transport and sorting in eukaryotic cells. Phospholipids synthesized in the endoplasmic reticulum (ER) are distributed symmetrically across the bilayer (green) due to a bidirectional flippase (1). The ER is connected with the Golgi via anterograde and retrograde vesicular pathways (arrows). Glycosphingolipids and sphingomyelin (SM) are synthesized on the lumenal aspect of the Golgi, where they aggregate together with cholesterol (red). They are excluded from retrograde transport vesicles to the ER, in contrast to unsaturated phosphatidylcholine (PtdCho) species (yellow) and are transported to the plasma membrane (arrow). Further lipid asymmetry is generated at the plasma membrane, possibly already in the Golgi, by an inward pumping aminophospholipid translocase and by outward pumping ABC transporters (2). Aminophospholipids hereby accumulate in the cytosolic bilayer leaflet (blue). Lipids are endocytosed by various carriers, probably starting from domains of the plasma membrane with different lipid compositions (arrow), to enter the endocytic recycling pathway, which might or might not include the Golgi (arrow). Some endocytic carriers become endosomes/lysosomes. A direct pathway from the Golgi shuttles lysosomal enzymes to the endosomes (arrow).

such anarchistic behaviour on its sophisticated transport system. We are beginning to find out how.

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Self-help for the glycophobic

Essentials of Glycobiology

edited by Ajit Varki, Richard Cummings, Jeffrey Esko, Hudson Freeze, Gerald Hart and Jamey Marth, Cold Spring Harbor Laboratory Press, 1999. $95.00 (653 pages) ISBN 0 87969 560 9

'That looks a thrilling read', quipped a passing graduate student as I wrestled this 650 page volume from *TCB*'s brown envelope. Why did I suspect sarcasm? Even the promotional material on the back of this book acknowledges that 'the prefix glyco strikes dread into the hearts of many molecular biologists.' However, many of these same biologists are being tempted, or even forced, to confront their glycophobia by the increasing number of studies that show that carbohydrate structures can have a key role in biological processes as diverse as protein folding, immune responses and development. This book is aimed at fulfilling a need for an introduction to the field of glycobiology that is both accessible to the outsider but sufficiently comprehensive and detailed to be worth reading by a wide range of scientists. The good news is that, by and large, the authors have succeeded in meeting this aim.

The book is based on a course for graduate students at UCSD, and this is reflected in a well-integrated structure. The traditional complaint about glycobiology is that it involves either complex carbohydrate chemistry or the enzymology of glycosyltransferases with names so long that they can extend for several lines of the title of a *JBC* paper. Determining the structure of carbohydrates is indeed a formidable challenge as, unlike proteins or nucleic acids, these polymers are often branched and can contain individual monomers with different structures but identical molecular masses. However, many of the basics of carbohydrate structure have been worked out for at least mammalian cells, and new techniques are speeding the analysis of structures in other organisms. In addition, the cloning of many genes for glycosyltransferases and for carbohydrate-binding proteins, combined with data from the genome projects, is now allowing carbohydrates to be examined as biological effectors in addition to simply being chemical structures.

The increasing interest in the biology of carbohydrates is reflected in the contents of this book being focused very strongly on biology, with every effort made to keep the chemical side both brief and accessible. Carbohydrate structures are shown as symbolic representations, and enzyme names are given in a simplified form. Despite the fact that colour is rarely used, presumably to ensure this large book is also financially accessible, the illustrations are clear, and the text is succinctly written in a readable style, although there are quite a few trivial errors. The book is also arranged in an approachable way with large themes broken into small bite-sized chapters, each ending with an extensive list of references and a discussion of future directions, the latter helping to convey a sense of the exciting potential of the field. The early chapters cover structure, nomenclature and synthesis of the glycans found on both proteins and lipids. The issue of biological function of glycans can be harder to discuss as they appear in such a broad range of systems, and yet in many cases their precise functions are poorly understood. The authors deal with this problem by initially outlining the general biological and evolutionary issues to be considered, including the important notion that at least some of the diversity of carbohydrate structures in a given organism might reflect a pressure to reduce adhesion by invading microbes, rather than a diversity of functions in the biology of the organism itself. When discussing the functions of a particular glycan, the authors clearly spell out what is speculation and what remains to be proven. A lot of emphasis is put on glycan-binding proteins – a valuable approach as it seems likely that, out of the vast array of carbohydrate structures, only those that have a particular protein to recognize them are likely to have important nonstructural roles. The book ends with chapters covering the role of glycans in genetic disorders and disease, and an account, perhaps too brief, of how glycan structures are analysed.

This book will be an invaluable introduction to the field for graduate students, undergraduates on specialist courses, and for biologists who have unexpectedly stumbled across a glycosyltransferase or carbohydrate-binding protein. It will also be a useful guide to biological issues for glycobiologists, although those wanting extensive details on carbohydrate

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