Transport and sorting of membrane lipids

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The lipid composition of cellular membranes may seem unnecessarily complex. However, the lipid composition of each membrane is carefully regulated by local metabolism and specificity in transport, marking the functional significance for the cell. Recent research has revealed unexpected discoveries concerning the topology of lipid synthesis, specificity in lipid transport, and the function of lipid and protein microdomains in sorting.

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Introduction

Each cellular membrane consists of a unique mixture of some 50 out of approximately 500 different membrane lipids, but the function of these complex lipid compositions has long remained obscure. The field of membrane lipid research has gained momentum by recent observations that specific glycolipids fulfil recognition events on the cell surface, that many common membrane lipids act as precursors for second messengers during signal transduction, and that various lipids function as membrane anchors for proteins. In addition, the lipid composition of the Golgi appears to be involved in the regulation of exocytotic vesicle transport.

Lipids diffuse in the plane of the membrane, across membrane bilayers and to some extent through the aqueous phase. In addition, vesicular pathways carry lipids between organelles of the vacuolar system. Without regulation, these processes would randomize the lipid composition of cellular membranes. A major challenge in cell biology is to find out how the cell succeeds in maintaining the differences in lipid composition between the various cellular membranes. This review begins with a description of sphingolipids, because most is known about their synthesis in the Golgi and their transport by membrane vesicles [1•–3•]. Cholesterol, thought to have a specific interaction with sphingolipids, is treated next [4•,5••]. Finally, the review deals with the synthesis and transport of the major phospho(glycero)lipids, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI). These lipids are synthesized at the cytosolic surface of the endoplasmic reticulum (ER), and although they are transported not only by vesicles but by exchange as well [6•], their trafficking displays specificity, like that of the sphingolipids and cholesterol. Our knowledge on the intracellular location of lipid synthesis and modification turns out to be fragmentary and superficial. The same is true for our insight into the relative contribution of the various transport mechanisms to the actual trafficking of individual lipid classes. By definition, therefore, ideas on specificity in lipid transport and the connection between lipid and protein sorting remain largely hypothetical.

Sites of sphingolipid synthesis

The precursor for all complex sphingolipids, ceramide, is synthesized in the ER [7•–9•], and is transported to the Golgi for conversion to sphingomyelin (SM) and glycosphingolipids, by an as yet unknown mechanism [10••,11•]. SM synthase transfers a phosphocholine headgroup from PC onto ceramide (Fig. 1). Studies that carefully discriminated Golgi from plasma membrane localized the major part of the SM synthase activity to the *cis*-Golgi. Thirteen per cent of the activity was found in the plasma membrane [12,13•]. In agreement with this, cells provided with radiolabeled precursors for phosphocholine [14•–16•] or for *de novo* synthesis of ceramide [10••,17•], synthesize radiolabeled SM mainly in the Golgi [16•].

The plasma membrane SM synthase [12], which was detected at the cell surface when ceramide was generated in the plasma membrane by external sphingomyelinase (SMase) [16•] is found on the basolateral surface of MDCK cells upon external addition of ceramide (ALB van Helvoort, W van 't Hof, T Ritsema, A Sandra, G van Meer, unpublished data). It may be different from the Golgi enzyme and could play an important role in signal transduction. We recently found that the well known lipid second messenger diacylglycerol (DG) [18•] is converted to PC on the MDCK basolateral surface and that this re-

Abbreviations

BFA—brefeldin A; **C_6-NBD**—N-6[7-nitro-2,1,3-benzoxadiazole-4-yl] aminohexanoyl; **DG**—diacylglycerol; **ER**—endoplasmic reticulum; **GalCer**—galactosylceramide; **GalNAc**—N-acetylgalactosamine; **GalT**—galactosyl transferase; **GlcCer**—glucosylceramide; **GlcT**—glucosyltransferase; **GPI**—glycosyl-phosphatidylinositol; **LacCer**—lactosylceramide; **LDL**—low-density lipoprotein; **PC**—phosphatidylcholine; **PE**—phosphatidylethanolamine; **PI**—phosphatidylinositol; **PS**—phosphatidylserine; **SM**—sphingomyelin.

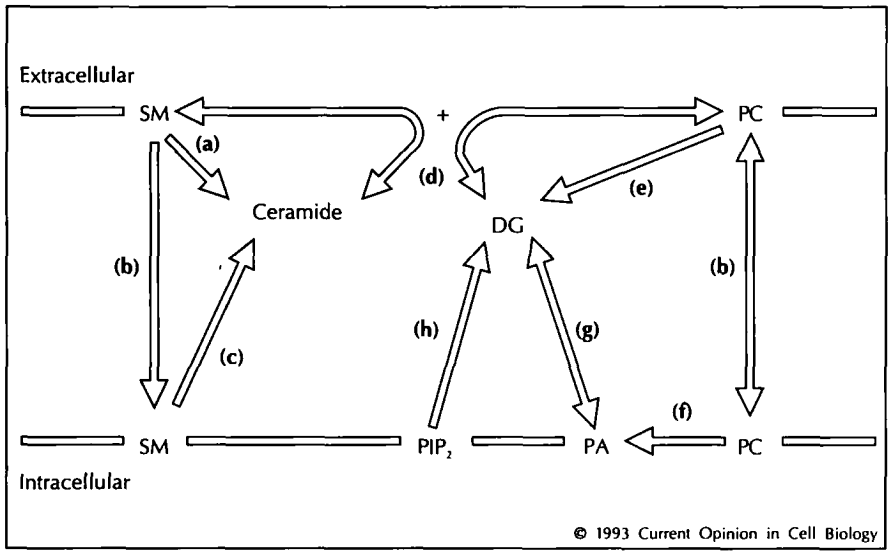

Fig. 1. Hydrolysis of plasma membrane phospholipids during signal transduction. The type of stimulus determines the kind of enzyme that will act: **(a)** neutral cell surface SMase [20,103], which would have to be regulated; **(b)** scrambling activity [52•,70••]; **(c)** neutral cytosolic SMase (Y Hannun, personal communication, [104]; because most, if not all, SM originally resides in the outer layer, possibly due to a cytosolic SMase, cell activation may induce SM translocation to the inner leaflet rather than switching on the SMase); **(d)** phosphocholine transferase (van Helvoort *et al.*, unpublished data); **(e)** exoplasmic PC–phospholipase C; **(f)** cytosolic phospholipase D; **(g)** DG kinase/ PA phosphohydrolase; **(h)** phosphoinositide–phospholipase C [18•].

action is inhibited by ceramide, another important lipid second messenger [19•]. The SM synthase, more commonly known as phosphocholine transferase, seems to interconvert the two second messengers on the cell surface (Fig. 1). The amounts of DG and ceramide that arise in the plasma membrane from hydrolysis of PIP_2, PC and SM upon stimulation of cells are significant. Values of 10–20 mol % of plasma membrane lipids are reported (references in [18•,19•]). Because DG and ceramide are non-bilayer forming lipids, dramatic (transient) changes in the organization and physical properties of the plasma membrane may ensue (Fig. 2). It remains unclear how DG and ceramide eventually disappear from the plasma membrane.

The location of SM synthase within the Golgi has not been completely resolved. Brefeldin A (BFA), which in most cells causes the Golgi (but not the *trans*-Golgi network) to merge into a mixed ER–Golgi, stimulated SM synthase several-fold [14•,15•,17•], confirming its location in early Golgi [12,13•,20]. By contrast, a late Golgi location was suggested by the observations that BFA inhibits SM synthesis in cerebellar cells [21] and that SM synthase does not relocate to the ER in otherwise BFA-sensitive HepG2 cells (Table 1) [22•].

Also, some of the first reactions in glycosphingolipid biosynthesis co-fractionated with *cis*-Golgi markers on sucrose gradients (Table 1): the transfer of glucose to ceramide by glucosyltransferase (GlcT) to yield glucosylceramide (GlcCer) [12,13•], and the sialyl transferases that convert Gal–GlcCer [lactosylceramide (LacCer)] to G_{M3} and G_{D3} [23•]. In the presence of BFA, these lipids and Lc3, a three sugar sphingolipid, were still produced [24•], suggesting redistribution of the respective transferases to the ER. Redistribution has been shown directly for GlcT [22•]. Whether the glycosyl transferases that no longer act on newly synthesized substrates are situated in the *trans*-Golgi network, which resists the action of BFA, remains to be clarified. Finally, the intra-Golgi localization of galactosyl transferase, GalT-1, that generates galactosylceramide (GalCer), is unknown.

Sidedness of sphingolipid synthesis in the Golgi

In intact Golgi membranes, SM synthase is protected against added proteases and newly synthesized SM is situated in the lumenal leaflet of the Golgi membrane [Fig. 3(a)]. In contrast, newly synthesized GlcCer is accessible at the cytosolic Golgi surface ([13•] and references therein). Although data from Trinchera *et al.* [25] suggest that LacCer is synthesized in the cytosolic leaflet of the Golgi as well, mutant Chinese hamster ovary cells lacking the UDP–galactose carrier but with normal levels of GalT-2 displayed strongly reduced LacCer synthesis, implying a lumenal GalT-2 disposition [26]. Indeed, newly synthesized short-chain LacCer is protected against extraction from the membrane by serum albumin (BSA). Surprisingly, just like GlcCer [13•], newly synthesized short-chain GalCer is extracted by BSA (KNJ Burger, P van der Bijl, G van Meer, unpublished data). Because GlcCer is galactosylated to LacCer and GalCer is sulfated to sulfatide in the lumenal leaflet of the Golgi, both GlcCer and GalCer have to translocate across the Golgi membrane (Fig. 1). Whether or not complex glycosphingolipids can translocate back to the cytosolic surface, where they could bind and thereby regulate specific cytosolic proteins, remains an important issue to be resolved [27•,28••,29•].

Sphingolipid transport and the occurrence of microdomains

The lumenal synthesis of both SM and the complex glycosphingolipids would predict that subsequent transport to the plasma membrane occurs by vesicles. Indeed, transport of LacCer between Golgi cisternae [30], and of G_{M3} [31••] and short-chain SMs [22•,32,33•] to the cell surface have been found to display typical characteristics of vesicular traffic. The cytosolic orientation of newly

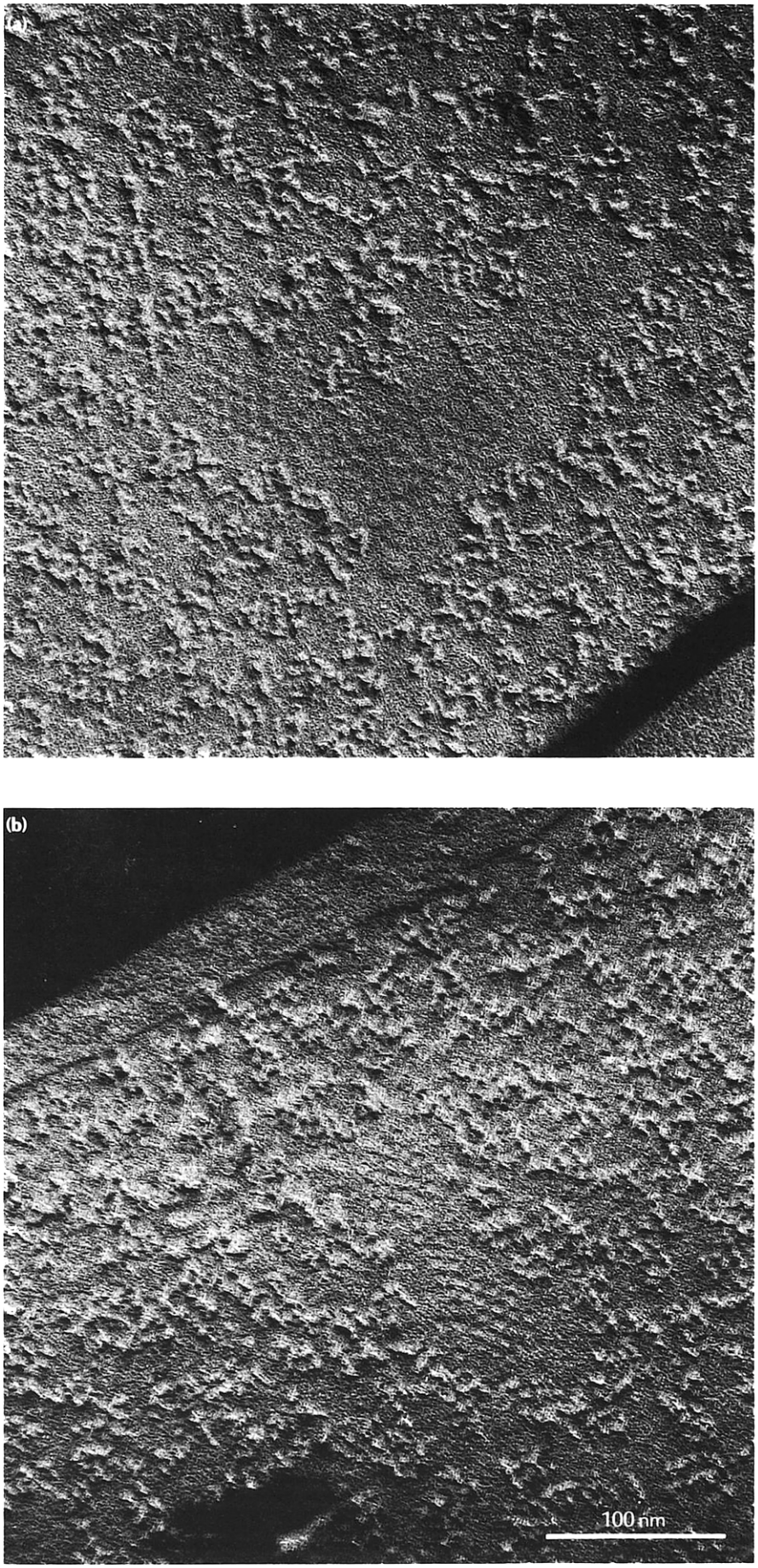

Fig. 2. Changes in plasma membrane organization due to the generation of non-bilayer lipids. Ceramide droplets in the hydrophobic core of the plasma membrane of a human erythrocyte after hydrolysis of 85 % of the SM (20 % of total phospholipid) with SMase, visualized by freeze fracture EM as 75 Å spheres on the exoplasmic fracture face **(a)** and complementary pits on the protoplasmic fracture face **(b)**. Direction of shadowing towards top of figure [105]. Segregation of DG has been reported upon treatment of membranes with phospholipase C at DG levels of 50 mol % [68], while DG remained intercalated in a (model) bilayer at < 10 mol % [106•].

Table 1. Distribution of SM synthase and glycosyltransferases over the Golgi stack.

Enzyme [1•]	Precursor [3•]	Product [3•]	Enzyme function with: BFA	Enzyme function with: Mitotic cells	Cell fractionation
SM synthase	Ceramide	SM	+ [14•,15•]	+ [10••]	*cis*/medial [12,13•]
GlcT	Ceramide	GlcCer	+ [21,24•,107]	+ [10••]	*cis* [12,13•]
GalT-2	GlcCer	LacCer	+ [21,24•,107]	+ [10••]	
SAT-1	LacCer	G_{M3}	+ [21,24•,107]		*cis* [23•,99]
SAT-2	G_{M3}	G_{D3}	+ [21,107]		*cis*/medial/*trans* [23•,99]
GlcNAcT	LacCer	Lc3	+ [24•]		*cis* [100]
GalNAcT	LacCer/G_{M3}, G_{D3}	G_{A2}/G_{M2}, G_{D2}	−[21,107]	−[10••]	medial [99]
GalT-6	LacCer	Gb3	−[24•]		
GalT-4	Lc3	nLc4	−[24•]		*trans* [100]
GalT-3	G_{A2}	G_{A1}			*trans* [99]
SAT-4/-5	G_{A1}/G_{M1b}	G_{M1b}/G_{D1c}			*trans* [23•,99]

For enzyme nomenclature see [1•]; only the substrates and products under study are listed (for a more complete overview see [3•]). These data support the idea that BFA would relocate enzymes from *cis*-Golgi back to the ER, and that the products can no longer reach the next glycosyltransferase. However, transport of (C_6-NBD-)SM and GlcCer to the plasma membrane was unaffected by BFA, SM synthase did not return to the ER [22•], and SM synthesis was reduced in cerebellar cells [21]. Also, GalNAcT, a medial-Golgi enzyme, no longer functioned with BFA, whereas it is generally believed that enzymes from the medial- and *trans*-Golgi, but not the *trans*-Golgi network, return to the ER.

synthesized GlcCer and GalCer would, in principle, allow transport of these lipids between organelles by protein-mediated exchange through the aqueous phase [3•]. However, three pieces of evidence currently favor a vesicular mechanism of transport. First, it has been reported that newly synthesized C_6-NBD–GlcCer, like C_6-NBD–SM, did not arrive at the surface of mitotic cells [34] where the normal vesicular Golgi route of lipid exocytosis appears to be inhibited [10••]. Second, transport of GlcCer and SM to the cell surface was reduced to the same extent by monensin [22•,35], by microtubule-depolymerization and low extracellular pH in epithelial cells (G van Meer, unpublished data) [22•], and by an inhibitor of sphingolipid synthesis [36•]. Finally, short-chain GlcCer was found to be partially protected in isolated Golgi [37,38•], and endocytosed C_6-NBD–GlcCer remained sequestered inside recycling organelles [39•].

It appears that sphingolipids follow all vesicular routes that exist in the cell. From the Golgi they reach the cell surface [16•,17•,22•,31••, 33•,36•], from where they recycle through early and late endosomes [20,39•] and finally end up in lysosomes where they are degraded [3•]. It is unclear at present to what extent they follow the direct pathway from Golgi to endosomes. After endocytosis, GlcCer has been observed to reach the Golgi [39•], and retrograde transport of sphingolipids through the Golgi stack and to the ER has been inferred from (re)utilization of endocytosed GlcCer for LacCer synthesis [40], the presence of endocytosed glycolipid Gb3, as monitored by its ligand Shiga toxin, through Golgi and ER [41••], and the presence of gangliosides and Forssman glycolipid in the ER and nuclear membrane [28••,42].

Sphingolipids do not partition into the various vesicular routes at random. In epithelial cells, newly synthesized short-chain GlcCer was enriched on the apical surface relative to SM [22•,33•,43], GalCer and sulfatide (P van der Bijl, M Lopes-Cardozo, G van Meer, unpublished data), which were preferentially delivered to the basolateral surface. Moreover, endocytosed C_6-NBD–GlcCer reached the Golgi, whereas C_6-NBD–SM, C_6-NBD–GalCer and C_6-NBD–LacCer preferentially recycled to the cell surface in (undifferentiated) epithelial HT29 cells [39•]. In most cell types, newly synthesized GlcCer reaches the cell surface earlier than SM [22•,37]. This may be due to a more efficient incorporation of GlcCer than of SM in anterograde transport vesicles, while, judging from the low GlcCer and SM concentration in the ER, both seem to prefer the anterograde route from *cis*-Golgi instead of the retrograde pathway to the ER. The only way by which a lumenal lipid can be concentrated into a specific vesicular pathway is by a relative increase in surface density at the site of vesicle budding. This implies the presence of at least two membrane domains that differ in lipid composition [Fig. 3(b)] [1•]. It has been demonstrated in innumerable model membrane studies that domain formation can be driven by simple lipid immiscibility and phase separation. However, lipid domains would only seem of biological relevance if the various domains could be recognized by specific proteins (reviewed in [44•]). Specific lipid and protein compositions have so far only been demonstrated for the macrodomains of the plasma membrane of epithelial and neuronal cells that in most cases are separated by a diffusion barrier [43,45•,46•]. The first example of specific lipids and proteins assembling into the same microdomain may be the combination of certain glycosphingolipids and a class of proteins attached to the exoplasmic side of the membrane by a glycosyl-phosphatidylinositol (GPI) anchor. In most epithelial cells, both glycosphingolipids and GPI

proteins are enriched on the apical cell surface relative to the basolateral surface. It has been proposed that glycosphingolipids are sorted in the *trans*-Golgi network by self-aggregation into apical precursor domains [43] and that GPI proteins preferentially associate with these putative glycosphingolipid microdomains [Fig. 3 (b)] [47••]. Indeed, Fischer rat thyroid (FRT) cells exhibit a reversed polarity of GPI proteins and epithelial lipid sorting also appears to be reversed [48••]. In addition, both GPI proteins and sphingolipids could be recovered from epithelial cells in detergent-insoluble membrane fragments, and newly synthesized GPI protein only became insoluble after entering the Golgi [47••]. The same result has been observed for an apical but not a basolateral transmembrane glycoprotein [49••]. Independently, the involvement of membrane microdomains in GPI protein sorting has recently been proposed, based on the observation that GPI proteins newly arrived at the apical surface were clustered and relatively immobile [50••]. On the cell surface, some GPI protein rich microdomains may persist, as GPI proteins are concentrated in caveolae that are also enriched in gangliosides (see Anderson, this issue, pp 647–652; [49••]). Interestingly, an integral caveolar membrane protein, caveolin/VIP21 has been localized to transport vesicles arising from the *trans*-Golgi network [49••]; in addition, tyrosine kinases on the cytosolic surface have been found associated with GPI proteins. Finally, it is important to note that both caveolar integrity and the detergent inextractability of GPI proteins were abolished by cholesterol depletion.

Cholesterol: intracellular distribution, site of synthesis and mechanism of transport

Although it is generally agreed that cholesterol concentration is highest in the plasma membrane and membranes of the endocytotic system, estimates of the actual fraction of cellular cholesterol residing in the plasma membrane can vary [4•,5••]. This is in part due to variation in the reported surface area of the plasma membrane, from 7 % of total membrane surface area for hepatocytes to 14 % for fibroblast-like baby hamster kidney (BHK) cells [51], or even 50 % for fibroblasts [4•]. An additional problem in the characterization of cholesterol transport is that little is known about the transbilayer distribution of cholesterol except that the half-time of its transbilayer movement is in the order of seconds [4•,52•]. The well known preferential interaction of cholesterol with SM, together with the exoplasmic orientation of SM [53,54•], predicts a dramatic enrichment of cholesterol in the outer leaflet of the plasma membrane. Unexpectedly, recent work assigned 75 % of erythrocyte cholesterol to the inner leaflet [55]. This controversy awaits to be resolved.

Although traditionally the ER has been considered the main site of cholesterol synthesis, recent evidence argues that peroxisomes are essential and may even be an obligatory stage during cellular cholesterol synthesis [56•]. As far as we know, peroxisomes are not connected to the vesicular transport routes, and as cholesterol has to reach the mitochondria of steroidogenic cells, cholesterol exchange through the cytosol must occur. The activity of carrier proteins for cholesterol in the cytoplasm is controversial. The non-specific lipid transfer protein, sterol carrier protein 2, is peroxisomal [57•,58•]. As the rate of both protein-mediated and spontaneous exchange strongly decreases with distance [6•], cholesterol exchange between peroxisomes, ER and mitochondria may require close contacts between the organelles. An indication for such a structural requirement is that efficient exchange depends on intact microfilaments and microtubules [5••]. This exchange and the mechanism by which cholesterol reaches the inner mitochondrial membrane closely parallel transport steps required for phospholipid transport (see below).

From the ER, cholesterol is transported to the plasma membrane predominantly by vesicular transport [4•,5••]. The persistence of this transport in the presence of BFA [59], also observed for PE [60], and short-chain SM and short-chain GlcCer [22•], has suggested a vesicular route that bypasses the Golgi [59]. However, as the Golgi has become part of the ER this route might simply reflect the Golgi–plasma membrane route. Still, it is surprising that lipid transport continues while transport has stopped for all proteins studied so far.

In addition, cholesterol derived from low-density lipoprotein (LDL)–cholesteryl esters in the lysosomes appears to reach other cellular membranes by vesicular traffic. Important information on this process has been obtained in extensive studies (reviewed in [5••]) demonstrating the incapability of LDL-derived cholesterol to reach regulatory sites in the ER in Niemann–Pick type C cells, in mutant cells [61•], in cells lacking intermediate filaments [62•], and in the presence of a number of lipophilic amines [63•], of progesterone [64•] and possibly mevinolin [65•]. The first step appears to be vesicular transport back to the plasma membrane, from where, most likely, retrograde transport through the Golgi leads to a rise in the ER cholesterol level. This finally activates esterification and downregulates cholesterol synthesis and uptake, by mechanisms that are not yet understood.

Whether cholesterol equilibrates between membranes by vesicular transport or by exchange, its steady-state distribution is most likely governed by its relative affinity for the components of the various membranes. The cholesterol concentration gradient from ER (low) to plasma membrane (high) parallels the SM content [53,66], and the sorting of SM (see above) may concentrate cholesterol into the exocytotic pathway. The retrograde transport of LDL-derived cholesterol through the Golgi against the proposed outward sorting by SM could simply reflect overloading of the sorting machinery. This idea is supported by the fact that lowering the SM content of the plasma membrane using SMase results in enhanced cholesterol transport to the ER [53]. Two other factors that contribute to the maintenance of the intracellular cholesterol gradient are the exchange equilibrium between the plasma membrane and extracellular lipoproteins, and the lowering of free cholesterol in the ER by esterification.

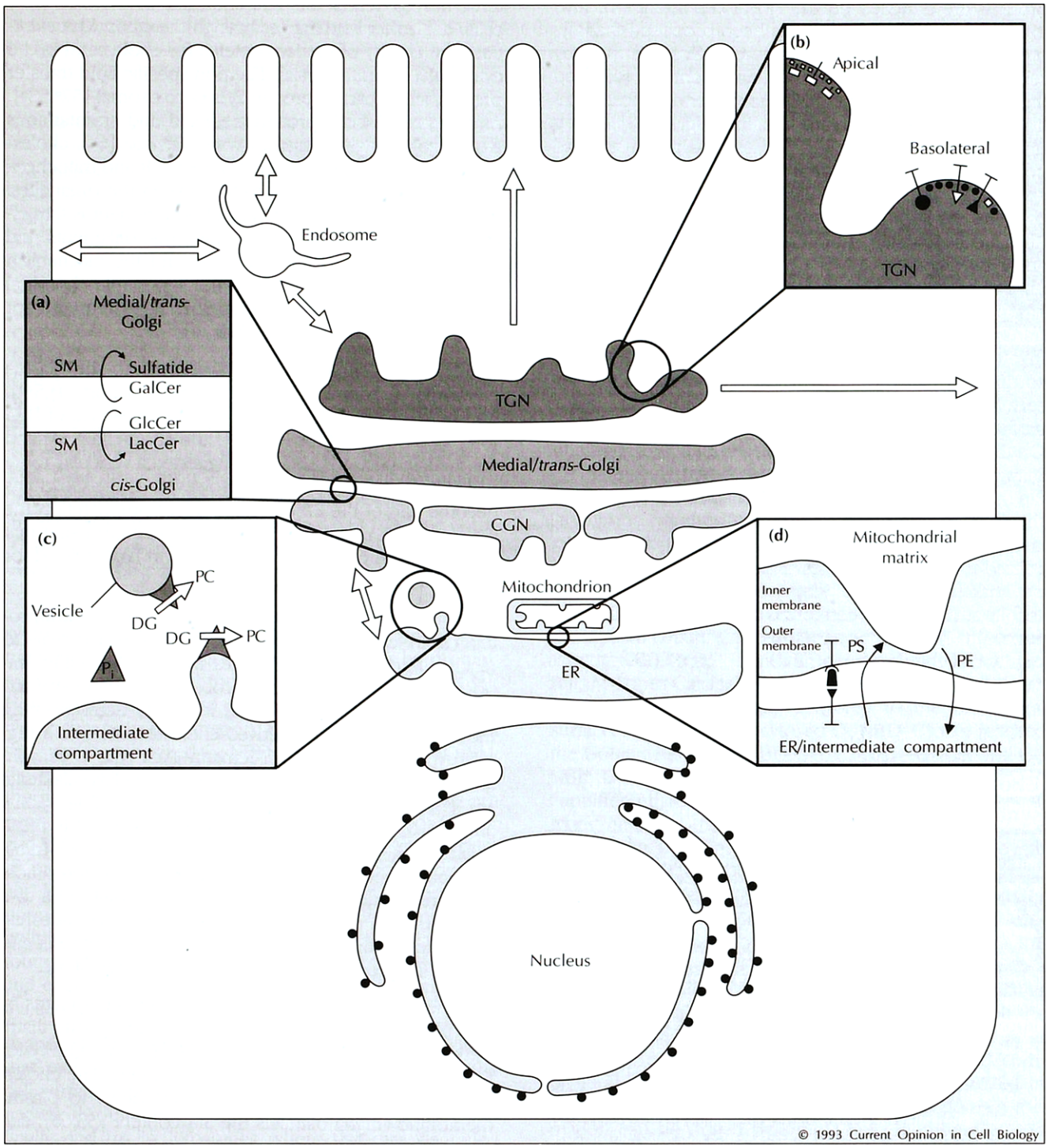

Fig. 3. Lipid trafficking in an epithelial cell. Arrows indicate vesicular pathways. **(a)** Sidedness of newly synthesized sphingolipids in the Golgi. **(b)** Defined sets of lipids and proteins are sorted to the apical and basolateral cell surface domains by preferential partitioning into either precursor domain. **(c)** PC is synthesized on the cytoplasmic surface of the ER. The enzyme that converts DG to PC is also present on the surface of transport vesicles to the Golgi. The phosphorylated inactive enzyme (P_i) is located in the cytosol. **(d)** After synthesis in a specialized part of the ER, closely associated with the mitochondrion (possibly the intermediate compartment), PS diffuses into and translocates across the outer mitochondrial membrane and is decarboxylated at the inner membrane. PE diffuses back to the ER. TGN, *trans*-Golgi network; CGN, *cis*-Golgi network.

Glycerophospholipid transport

The glycerophospholipids PC, PE, PS and PI are synthesized at the cytosolic surface of the ER by a number of pathways. The relative contribution of each depends on cell type [67•]. They can rapidly equilibrate over the ER membrane by lateral diffusion and by facilitated diffusion across the membrane through the action of a non-spe-

cific flippase ($t_{1/2}$ = 20 min) [52•,68]. The glycerophospholipids are the main constituents of the membrane of the transport vesicles that travel between the organelles of the vacuolar system. Because they also constitute the bulk of the lipids of the mitochondrial and peroxisomal membranes, they must travel by a different mechanism as well, presumably by monomeric exchange. What, then, imposes specificity onto glycerophospholipid transport?

In the plasma membrane, the endocytotic pathway and, presumably in the Golgi, the phospholipids do not equilibrate rapidly across the bilayer. An asymmetric phospholipid distribution is maintained by the aminophospholipid translocase, an ATP-dependent enzyme that pumps aminophospholipids (PS and with less efficiency PE) towards the cytoplasmic side, possibly in combination with other pump proteins [52•,69••]. As a result, the cytosolic leaflet of these membranes contains essentially all PS, most PE (and PI), a little less than half of the PC and essentially no SM. The asymmetrical arrangement of the lipids may deteriorate during fusion and fission reactions, passage through the ER, or during cell activation [70••], in the latter case by a regulated Ca^{2+}-activated scramblase [52•] or by a physical disruption of plasma membrane structure (Fig. 1). The combined action of lipid pumps may repair such losses in asymmetry.

The mechanism by which PC is transported through the cell depends on its localization. After synthesis, PC can flip across the ER membrane and follow the vesicular pathway to the cell surface from where it can be endocytosed and recycled, idling away in the lumenal leaflet. From the ER, PC could also follow the vesicular pathways on the cytosolic face of the membranes. The fact that PC synthesis seems to continue on the transport vesicles that travel from the ER to the Golgi (possibly the intermediate compartment) corroborates the notion that such synthesis may provide the curvature needed for budding in the ER [Fig. 3(c)] [71•]. At the same time, being oriented towards the cytosol, PC could reach other membranes by monomeric exchange. Within minutes, newly synthesized PC reaches the plasma membrane [72] and equilibrates with PC in the outer mitochondrial membrane [73]. This strongly suggests the involvement of transfer proteins present in the cytosol [74•]. It should be noted that, so far, the potential of these proteins to support a net transfer of phospholipids under biologically relevant conditions has not been demonstrated.

Interestingly, newly synthesized PE appears at the cell surface [34,60] and equilibrates with the outer mitochondrial membrane [73], at an order of magnitude slower than PC with a typical half-time of 0.5–1.5 h. Whereas PE may reach the surface by a combination of either vesicular traffic or exchange with flip-flop, transport to the mitochondrion most likely involves monomeric exchange. PE still reaches the surface during mitosis [34] and in the presence of BFA [60]. This could suggest transport through monomeric exchange, but it should be realized that for some lipids a vesicular pathway persisted in the presence of BFA [22•,59].

PI, the precursor for GPI-anchored proteins [47••] and for the signal transduction phosphoinositides PIP, PIP_2 and PIP_3, is synthesized in the ER [75,76,77•]. PI kinase and PIP kinase are distributed along the exocytotic route [75] and in the nucleus [75,78•]. While PI is found in all cellular membranes, PIP_2 is especially abundant in the plasma membrane and, in addition, in the nucleus in a form that is not membrane-associated [28••]. The presence of regulated PIP_2–phospholipase C (and protein kinase C) activities both on the inside of the plasma membrane and in the nucleus have led to the suggestion that separate plasma membrane and intranuclear inositide cycles exist [78•]. In the plasma membrane, the phosphoinositides have been found preferentially in the cytoplasmic leaflet [52•], and the distribution of PI may be maintained by an exoplasmic PI–phospholipase C [79]. Phosphoinositides can thus be transported on the cytosolic face of carrier vesicles. In addition, PI may exchange between membranes mediated by the cytoplasmic PI–PC transfer protein [74•] or not [80•]. A sensational development is the apparent role of this protein (it is essential for growth of yeast) and of PI kinase in the exocytotic pathway, where they appear to be involved in regulation of vesicular transport and sorting. While the PI–PC transfer protein may monitor and/or modify the PI:PC ratio in the cytosolic leaflet of the Golgi [74•], the PI kinase appears to function in a signal transduction complex (see Stack and Emr, this issue, pp 641–646).

Sorting of glycerophospholipids

There are some indications that microdomain formation in vesicular pathways may also be involved in sorting glycerophospholipids. Because the sphingolipids are preferentially included in certain routes, lumenal PC could be incorporated into other ones by default, e.g. from the *trans*-Golgi network to the basolateral cell surface of epithelial cells, which is indeed enriched in PC [43], and into the retrograde pathway through the Golgi to the ER [81]. Sorting by microdomain formation may occur in cytosolic leaflets as well: the enrichment of PS in the plasma membrane [82] could be explained by anterograde sorting of PS in the Golgi. However, PS is possibly sorted in a different way. While short-chain C_6-NBD–PS after translocation across the plasma membrane diffused all over the cell, the less readily exchangeable C_{12}-NBD–PS, after insertion into the cell surface and ATP-dependent flip-flop, concentrated in the Golgi by what seemed to be an exchange process as it was functional in mitotic cells [83].

There is evidence that newly synthesized lipids do not freely intermix with the pre-existing pool of that lipid in the ER [71•,76,82,84–86,87•,88•]. PS is synthesized by exchanging the choline headgroup of PC or the ethanolamine headgroup of PE for serine, mostly in the ER [89]. Newly synthesized PS transported to the plasma membrane [82] and to mitochondria [80•,86,88•] has a higher specific radioactivity than PS in ER, and it has been proposed that PS is synthesized in a specialized domain of the ER that is in close physical contract with the

outer mitochondrial membrane [Fig. 3(d)]. The need for contact could explain the ATP (or Ca^{2+}) requirement of PS transport [90••]. Indeed, upon cell fractionation, a mitochondrion-associated membrane displayed a high specific activity of PS synthase [85], PS transport in permeabilized cells was insensitive to dilution of the assay system [90••] and PS transport from ER to mitochondria occurred *in vitro*, but not to mitochondria isolated from separate cells [90••]. The advantages of close contacts for exchange processes have been discussed in detail by Brown [6•]. After reaching the outer mitochondrial membrane, all evidence indicates that PS rapidly flip-flops and reaches the PS decarboxylase in the inner membrane by lateral diffusion most likely through the contact sites between the inner and outer membranes [91,92••,93••,94•]. The resulting PE does not mix with the inner membrane PE and is rapidly exported to the mitochondrion-associated ER. This compartmentalization is again not topologically understood. In the mitochondrion-associated membrane, PS and PC are synthesized and PS-derived PE is received from the mitochondrion [85,86]. The preferential transport of PC and PS-derived PE from this site to lipoproteins in the Golgi lumen [84] and of PC and PS [82] and PS-derived PE [60] to the plasma membrane suggests that this membrane is part of the exocytotic route. Possibly it belongs to the smooth, peripheral elements of the intermediate compartment [71•,95•].

Conclusion

Progress in the field of lipid transport and sorting may now be expected from careful localization of the proteins involved in the synthesis, hydrolysis and transport of lipids and from defining the sidedness of these events. In the end the cytoskeleton will also turn out to be an important organizer of transport. In addition, quantitative analysis of the intracellular lipid distribution and accurate definition of the kinetic parameters of the various processes will be of great help in finding regulatory hot-spots and defining the proteins involved.

Finally, we are only starting to realize what physical properties of lipids may be important for cellular functions. What makes lipids segregate into domains and how are these domains recognized by proteins? What makes a membrane bend and bud? What defines the size of organelles? What sensor measures the concentration of the various lipids in each membrane, and how does it feed back to the responsible enzymes and genes? It is gratifying to see how many different fields of science now merge to contribute to a solution to these integrated questions, questions of a living cell.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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This review contains a discussion on transport assays and theoretical notes on the relation between pathway sizes and lipid sorting, applied to the sorting of sphingolipids by aggregation into microdomains.

2. • HOEKSTRA D, KOK JW: **Trafficking of Glycosphingolipids in Eukaryotic Cells; Sorting and Recycling of Lipids.** *Biochim Biophys Acta* 1992, 1113:277–294.

An overview of studies of glycosphingolipid synthesis, vesicular traffic and sorting.

3. • VAN ECHTEN G, SANDHOFF K: **Ganglioside Metabolism. Enzymology, Topology and Regulation.** *J Biol Chem* 1993, 268:5341–5344.

Reviews ganglioside biosynthesis in ER/Golgi and hydrolysis in the lysosomes with the help of activator proteins, some of which act as glycolipid transfer proteins *in vitro*.

4. • LANGE Y: **Tracking Cell Cholesterol with Cholesterol Oxidase.** *J Lipid Res* 1992, 33:315–321.

The results of this study should be interpreted with caution, because the method is invasive and the oxidase changes the structure of the membrane under study.

5. •• LISCUM L, DAHL NK: **Intracellular Cholesterol Transport.** *J Lipid Res* 1992, 33:1239–1254.

An extensive overview of the progress in studying cholesterol transport. The number of persisting discrepancies illustrates the practical difficulties of assaying the intracellular localization of cholesterol at a defined point in time.

6. • BROWN RE: **Spontaneous Lipid Transfer Between Organized Lipid Assemblies.** *Biochim Biophys Acta* 1992, 1113:375–389.

Features the transient collision model for spontaneous lipid transfer as opposed to the hitherto dominating model of lipid monomer diffusion and to the model of hemifusion. It predicts that significant exchange of most biological lipids will only occur when the membranes are very closely apposed. The close apposition of the target membrane enhances the desorption of lipid monomers from the donor membrane, after which they diffuse across the aqueous phase.

7. • MANDON EC, EHSES I, ROTHER J, VAN ECHTEN G, SANDHOFF K: **Subcellular Localization and Membrane Topology of Serine Palmitoyltransferase, 3-Dehydrosphinganine Reductase, and Sphinganine N-Acyltransferase in Mouse Liver.** *J Biol Chem* 1992, 267:11144–11148.

Dihydroceramide is synthesized on the cytosolic face of purified ER membranes. Proteases inactivated the enzymes by 60–80% under conditions where glucose-6-phosphatase remained latent towards mannose-6-phosphate.

8. • ROTHER J, VAN ECHTEN G, SCHWARZMANN G, SANDHOFF K: **Biosynthesis of Sphingolipids: Dihydroceramide and not Sphinganine is Desaturated by Cultured Cells.** *Biochem Biophys Res Commun* 1992, 189:14–20.

The introduction of the double bond in the sphingolipid backbone only occurs after acylation of sphinganine: dihydroceramide is converted to ceramide. Desaturated sphinganine, sphingosine, is not a biosynthetic intermediate but exclusively a catabolic product of cellular sphingolipids.

9. HIRSCHBERG K, RODGER J, FUTERMAN AH: **The Long-Chain Sphingoid Base of Sphingolipids is Acylated at the Cytosolic Surface of the Endoplasmic Reticulum in Rat Liver.** *Biochem J* 1993, 290:751–757.
Sphinganine-N-acyltransferase faces the cytosolic side of purified ER membrane as established by limited pronase digestion under conditions where mannose-6-phosphatase remained latent.

10. COLLINS RN, WARREN G: **Sphingolipid Transport in Mitotic HeLa Cells.** *J Biol Chem* 1992, 267:24906–24911.
In mitotic HeLa cells, where vesicular traffic is inhibited, synthesis of GlcCer and LacCer is approximately normal while SM synthesis is reduced by 30 %. Apparently, ceramide still reached the Golgi, or Golgi enzymes were relocated to the ER [96•] as with BFA. As with BFA [24•], GalNAc addition to LacCer to yield G_{A2} no longer occurred, suggesting that LacCer no longer reaches the GalNAcT compartment.

11. MOREAU P, CASSAGNE C, KEENAN TW, MORRÉ DJ: **Ceramide Excluded from Cell-Free Vesicular Lipid Transfer from Endoplasmic Reticulum to Golgi Apparatus — Evidence for Lipid Sorting.** *Biochim Biophys Acta* 1993, 1146:9–16.
After vortexing with ER isolated from rat liver, [^{3}H]-ceramide was not found to be transported to isolated Golgi by transition vesicles. Conversion to higher sphingolipids was not monitored.

12. FUTERMAN AH, PAGANO RE: **Determination of the Intracellular Sites and Topology of Glucosylceramide Synthesis in Rat Liver.** *Biochem J* 1991, 280:295–302.

13. JECKEL D, KARRENBAUER A, BURGER KNJ, VAN MEER G, WIELAND F: **Glucosylceramide is Synthesized at the Cytosolic Surface of Various Golgi Subfractions.** *J Cell Biol* 1992, 117:259–267.
In intact Golgi membranes GlcT is sensitive to proteases and non-membrane permeant protein modifying reagents [12,25,97]. SM synthase is insensitive [12]. After synthesis, two short-chain GlcCer analogs are accessible on the cytosolic surface of isolated Golgi membranes and in permeabilized cells. Newly synthesized SM remained inaccessible in the lumenal leaflet of the Golgi membrane [32]. GlcT activity showed two distinct peaks after subfractionation of the Golgi on sucrose gradients. C_6-NBD–ceramide was not found enriched in Golgi fractions [35].

14. BRÜNING A, KARRENBAUER A, SCHNABEL E, WIELAND FT: **Brefeldin A-Induced Increase of Sphingomyelin Synthesis.** *J Biol Chem* 1992, 267:5052–5055.
In Chinese hamster ovary cells, BFA increased SM synthesis from [^{3}H]-choline twofold, and synthesis of SM (but not GlcCer) from exogenous C_8/C_8–ceramide fivefold. Transport of C_8/C_8–SM out of the cell was reduced. At the same time Linardic *et al.* [98•] reported that BFA caused hydrolysis of a fixed pool of 20–25 % of prelabeled SM in HL60 cells within 5–20 min.

15. HATCH GM, VANCE DE: **Stimulation of Sphingomyelin Biosynthesis by Brefeldin A and Sphingomyelin Breakdown by Okadaic Acid Treatment of Rat Hepatocytes.** *J Biol Chem* 1992, 267:12443–12451.
BFA stimulated SM synthesis from natural precursors approximately twofold. Okadaic acid induced the catabolism of newly synthesized SM, suggesting a SMase regulated by phosphorylation.

16. KALLEN K-J, QUINN P, ALLAN D: **Monensin Inhibits Synthesis of Plasma Membrane Sphingomyelin by Blocking Transport of Ceramide Through the Golgi: Evidence for Two Sites of Sphingomyelin Synthesis in BHK Cells.** *Biochim Biophys Acta* 1993, 1166:305–308.
External SMase is used as an assay for cell surface SM (not an ideal assay, as SMase modifies the membrane under study; Fig. 2; cf. [4•]). Previously, the authors showed that upon addition of [^{3}H]-choline the specific activity of surface SM lagged behind that of intracellular SM by 2 h. Either newly synthesized SM slowly equilibrates with surface SM or a small pool of new SM feeds into a large pool of surface SM unidirectionally. Monensin reduces SM synthesis from [^{3}H]-choline–PC, but not from C_6-NBD–ceramide [22•,35]. Part of the SM synthase may no longer be reached by natural ceramide. Transport of SM synthesized in the presence of monensin to the cell surface is severely reduced [22•,35]. Surface SM degraded by SMase was resynthesized on the surface as defined by its sensitivity to a second SMase treatment and insensitivity to monensin.

17. KALLEN K-J, QUINN P, ALLAN D: **Effects of Brefeldin A on Sphingomyelin Transport and Lipid Synthesis in BHK21 Cells.** *Biochem J* 1993, 289:307–312.
BFA increased synthesis of SM and GlcCer (and cholesteryl ester) from [^{3}H]-acetate. Transport of [^{3}H]-SM to the cell surface was not observed. The increased cholesterol ester synthesis, which has also been reported by others, could reflect reduced SM transport to the cell surface. Reducing surface SM by treatment of intact cells with SMase has the same effect [53].

18. LISCOVITCH M: **Crosstalk Among Multiple Signal-Activated Phospholipases.** *Trends Biochem Sci* 1992, 17:393–399.
Review on the production of lipid second messengers produced by phospholipases C, D and A_2 at the plasma membrane and their role in signaling.

19. KOLESNICK R: **Ceramide: a Novel Second Messenger.** *Trends Cell Biol* 1992, 2:232–236.
Reviews the rapidly developing field of sphingolipid-derived second messengers.

20. KOVAL M, PAGANO RE: **Intracellular Transport and Metabolism of Sphingomyelin.** *Biochim Biophys Acta* 1991, 1082:113–125.

21. VAN ECHTEN G, IBER H, STOTZ H, TAKATSUKI A, SANDHOFF K: **Uncoupling of Ganglioside Biosynthesis by Brefeldin A.** *Eur J Cell Biol* 1990, 51:135–139.

22. VAN MEER G, VAN 'T HOF W: **Epithelial Sphingolipid Sorting is Insensitive to Reorganization of the Golgi by Nocodazole, but is Abolished by Monensin in MDCK Cells and by Brefeldin A in Caco-2 Cells.** *J Cell Sci* 1993, 104:833–842.
In HepG2 cells, BFA redistributed glucosyltransferase to the ER, but not SM synthase. Transport of C_6-NBD–SM and C_6-NBD–GlcCer to the cell surface was unchanged. In the epithelial Caco-2 cells, BFA abolished sorting between the two lipids and they reached the cell surface with equal apical : basolateral ratios. Monensin had a similar effect in MDCK cells. The microtubule-depolymerizing agent nocodazole selectively inhibited apical transport of both lipids to the same extent (by 50 %), strongly suggesting that SM and GlcCer are transported to the apical cell surface by the same, vesicular pathway. No missorting of apical lipids to the basolateral cell surface was observed.

23. IBER H, VAN ECHTEN G, SANDHOFF K: **Fractionation of Primary Cultured Cerebellar Neurons: Distribution of Sialyltransferases Involved in Ganglioside Biosynthesis.** *J Neurochem* 1992, 58:1533–1537.
Whereas the activity of sialytransferase I (SAT-1, which gives G_{M3}) decreases along the *cis–trans*-Golgi axis, that of the late sialytransferases SAT-4 and SAT-5 increases, while SAT-2 (which gives G_{D3}) was evenly distributed. Very similar data have been reported for liver Golgi [99]. In addition, GalNAcT (giving G_{A2}, G_{M2} and G_{D2}) co-localized with medial-Golgi and GalT-3 (giving G_{A1}, G_{M1} and G_{D1b}) with a *trans*-Golgi marker.

24. SHERWOOD AL, HOLMES EH: **Brefeldin A Induced Inhibition of *de novo* Globo- and Neolacto-Series Glycolipid Core Chain Biosynthesis in Human Cells.** *J Biol Chem* 1992, 267:25328–25336.
BFA did not inhibit addition of sialic acid or GlcNAc to LacCer (SAT-1 giving G_{M3} and GlcNAcT giving Lc3). BFA blocked galactose addition to LacCer (GalT-6 giving Gb3) and to Lc3 (GalT-4 giving nLc4). Indeed, sucrose gradient fractionation located 50 % of GlcNAcT to a more proximal compartment than the bulk of GalT-4 [100]. Earlier BFA studies localized GlcT, GalT-2, SAT-1 and SAT-2 into the BFA-sensitive compartment and GalNAcT past the BFA block (Table 1).

25. TRINCHERA M, FABBRI M, GHIDONI R: **Topography of Glycosyltransferases Involved in the Initial Glycosylations of Gangliosides.** *J Biol Chem* 1991, 266:20907–20912.

26. DEUTSCHER SL, HIRSCHBERG CB: **Mechanism of Galactosylation in the Golgi Apparatus. A Chinese Hamster Ovary Cell Mutant Deficient in Translocation of UDP-Galactose Across Golgi Vesicle Membranes.** *J Biol Chem* 1986, 261:96–100.

27. HIGASHI H, OMORI A, YAMAGATA T: **Calmodulin, a Ganglioside-Binding Protein.** *J Biol Chem* 1992, 267:9831–9838.
Calmodulin was isolated from cytosol by a ganglioside affinity column. Other ganglioside-binding proteins had been isolated from cytoplasm before and small amounts of gangliosides have been reported

in cytosolic fractions. Whether the observed *in vitro* modulation of a calmodulin-dependent cyclic nucleotide phosphodiesterase by binding of gangliosides is of physiological relevance depends on whether gangliosides are expressed on cytosolic surfaces.

28. •• VOORHOUT WF, VAN GENDEREN IL, YOSHIOKA T, FUKAMI K, GEUZE HJ, VAN MEER G: **Subcellular Localization of Glycolipids as Revealed by Immuno-Electronmicroscopy.** *Trends Glycosci Glycotechnol* 1992, 4:533–546.

Summarizes earlier work on the localization of the complex glycosphingolipid Forssman antigen in MDCK cells. The lipid was found in all membranes connected by vesicular traffic, most prominently the plasma membrane and endocytotic membranes, but not in mitochondria and peroxisomes. The signal transduction lipid PIP_2 was localized to the plasma membrane. However, a second monoclonal antibody yielded intense PIP_2 labeling over the nuclear matrix, suggesting the presence of PIP_2–protein complexes and corroborating the presence of a complete phosphoinositide signal transduction cascade in the nucleus [78•].

29. • GILLARD BK, THURMON LT, MARCUS DM: **Variable Subcellular Localization of Glycosphingolipids.** *Glycobiology* 1993, 3:57–67.

In fixed digitonin-permeabilized cells, glycosphingolipids (e.g. GalCer and G_{M1}) display dissimilar distributions by immuno-fluorescence. If no redistribution occurred during permeabilization (difficult to prove), the two lipids would have been sorted (after Golgi synthesis) to different locations. Polysialo-gangliosides would be present in mitochondria. Although 12 % of the gangliosides in rat liver were found in mitochondrial cell fractions [42], no Forssman glycolipid was found in mitochondria by a non-invasive technique [28••]. By using similar detergent methods the authors observed glycosphingolipid association to intermediate filaments. Labeling could reflect glycosphingolipid containing vesicles, or glycosphingolipid monomers bound to intermediate filaments before or during permeabilization.

30. WATTENBERG BW: **Glycolipid and Glycoprotein Transport Through the Golgi Complex are Similar Biochemically and Kinetically. Reconstitution of Glycolipid Transport in a Cell Free System.** *J Cell Biol* 1990, 111:421–428.

31. •• YOUNG WW JR, LUTZ MS, BLACKBURN WA: **Endogenous Glycosphingolipids Move to the Cell Surface at a Rate Consistent with Bulk Flow Estimates.** *J Biol Chem* 1992, 267:12011–12015.

Labeled G_{M3} reached the plasma membrane of Chinese hamster ovary cells 5–6 min after synthesis as measured by oxidation of cell surface ganglioside with periodate. The first use of this method showed that transfer of gangliosides from the site of synthesis to the plasma membrane of neuroblastoma cells required some 20 min, a process blocked at 15°C [101].

32. HELMS JB, KARRENBAUER A, WIRTZ KWA, ROTHMAN JE, WIELAND FT: **Reconstitution of Steps in the Constitutive Secretory Pathway in Permeabilized Cells. Secretion of Glycosylated Tripeptide and Truncated Sphingomyelin.** *J Biol Chem* 1990, 265:20027–20032.

33. • VAN 'T HOF W, SILVIUS J, WIELAND F, VAN MEER G: **Epithelial Sphingolipid Sorting Allows for Extensive Variation of the Fatty Acyl Chain and the Sphingosine Backbone.** *Biochem J* 1992, 283:913–917.

Sorting was defined [43] as a difference in apical/basolateral polarity of arrival at the cell surface between GlcCer and SM, after synthesis from added short-chain ceramide. GlcCer was apically enriched over SM, twofold to sixfold in MDCK and threefold to ninefold in Caco-2 cells. Sorting efficiency, but not direction, was influenced by variations in the fatty acyl chain or the sphingosine backbone. Even GlcCer synthesized from the water-soluble truncated C_8/C_8–ceramide [37] had an apical/basolateral polarity threefold higher than SM.

34. KOBAYASHI T, PAGNO RE: **Lipid Transport During Mitosis.** *J Biol Chem* 1989, 264:5966–5973.

35. LIPSKY NG, PAGANO RE: **Intracellular Translocation of Fluorescent Sphingolipids in Cultured Fibroblasts: Endogenously Synthesized Sphingomyelin and Glucocerebroside Analogues Pass Through the Golgi Apparatus en route to the Plasma Membrane.** *J Cell Biol* 1985, 100:27–34.

36. • ROSENWALD AG, PAGANO RE: **Inhibition of Glycoprotein Traffic Through the Secretory Pathway by Ceramide.** *J Biol Chem* 1993, 268:4577–4579.

Transport of vesicular stomatitis virus G protein through the Golgi complex was progressively retarded by increasing concentrations of ceramide, similar to the previously reported effect of PDMP, an inhibitor of GlcCer (and SM) synthesis. The ceramide concentrations applied were far higher than those normally used in short-chain sphingolipid transport studies [22•,33•,34,35,38•,43].

37. KARRENBAUER A, JECKEL D, JUST W, BIRK R, SCHMIDT RR, ROTHMAN JE, WIELAND FT: **The Rate of Bulk Flow from the Golgi to the Plasma Membrane.** *Cell* 1990, 63:259–267.

38. • KOBAYASHI T, PIMPLIKAR SW, PARTON RG, BHAKDI S, SIMONS K: **Sphingolipid Transport from the Trans-Golgi Network to the Apical Surface in Permeabilized MDCK Cells.** *FEBS Lett* 1992, 300:227–231.

C_6-NBD–GlcCer and C_6-NBD–SM, like C_8/C_8–GlcCer and C_8/C_8–SM [37], were found to be protected against BSA back-exchange in isolated vesicles.

39. • KOK JW, HOEKSTRA K, ESKELINEN S, HOEKSTRA D: **Recycling Pathways of Glucosylceramide in BHK Cells: Distinct Involvement of Early and Late Endosomes.** *J Cell Sci* 1992, 103:1139–1152.

The vesicular pathway from both early and late endosomes back to the cell surface was followed by C_6-NBD–GlcCer. Earlier work demonstrated that N-rhodamine–PE was efficiently transported to lysosomes in BHK, whereas C_6-NBD–GlcCer was routed to the Golgi and C_6-NBD–SM and C_6-NBD–LacCer were efficiently recycled in undifferentiated HT29 cells.

40. TRINCHERA M, CARRETTONI D, GHIDONI R: **A Part of Glucosylceramide Formed from Exogenous Lactosylceramide is not Degraded to Ceramide but Re-Cycled and Glycosylated in the Golgi Apparatus.** *J Biol Chem* 1991, 266:9093–9099.

41. •• SANDVIG K, GARRED Ø, PRYDZ K, KOZLOV JV, HANSEN SH, VAN DEURS B: **Retrograde Transport of Endocytosed Shiga Toxin to the Endoplasmic Reticulum.** *Nature* 1992, 358:510–512.

In A431 cells treated with butyric acid, Shiga toxin reaches the ER and nuclear envelope. The authors showed globotriaosylceramide (Gb3) to be the receptor for Shiga toxin.

42. MATYAS GR, MORRÉ DJ: **Subcellular Distribution and Biosynthesis of Rat Liver Gangliosides.** *Biochim Biophys Acta* 1987, 921:599–614.

43. SIMONS K, VAN MEER G: **Lipid Sorting in Epithelial Cells.** *Biochemistry* 1988, 27:6197–6202.

44. • JACOBSON K, VAZ WLC: **Domains in Biological Membranes.** *Comm Mol Cell Biophys* 1992, 8:1–114.

Discusses the occurrence of domains in biomembranes, measurements of their size and lifespan, and possible biological functions.

45. • KOBAYASHI T, STORRIE B, SIMONS K, DOTTI CG: **A Functional Barrier to Movement of Lipids in Polarized Neurons.** *Nature* 1992, 359:647–650.

Using methodology previously applied to epithelial cells [43], fluorescent phospholipid (N-NBD–PE) was fused into the axonal plasma membrane. Fluorescence recovery after photobleaching was measured to show that the lipids were mobile. No label was observed on cell body or dendrites for 60 min independent of whether the lipid was present in the outer or the inner leaflet of the plasma membrane. A lipid diffusion barrier at the axonal hillock (in both membrane leaflets) was proposed [46•].

46. • FUTERMAN AH, KHANIN R, SEGEL LA: **Lipid Diffusion in Neurons.** *Nature* 1993, 362:119.

Based on model calculations, the authors question the need for a physical diffusion barrier to maintain differences in lipid composition between neuronal axons and the cell body.

47. •• BROWN DA, ROSE JK: **Sorting of GPI-Anchored Proteins to Glycolipid-Enriched Membrane Subdomains During Transport to the Apical Cell Surface.** *Cell* 1992, 68:533–544.

After entering the Golgi, GPI proteins were resistant to TX-100 extraction from MDCK cells in the cold. After the extraction, both GPI

proteins and sphingolipids were recovered in the same vesicles. As it is unclear how TX-100 extracts lipids from an asymmetric membrane and as virtually all SM (a large part of which is in the basolateral plasma membrane domain) ends up in the TX-100 resistant fraction, the data do not prove that GPI proteins and sphingolipids were in close contact before the detergent was added. The study does demonstrate that sphingolipids and GPI proteins share physicochemical properties.

48. •• ZURZOLO C, LISANTI MP, CARAS IW, NITSCH L, RODRIGUEZ-BOULAN E: **Glycosylphosphatidylinositol-Anchored Proteins are Preferentially Targeted to the Basolateral Surface in Fischer Rat Thyroid Epithelial Cells.** *J Cell Biol* 1993, 121:1031–1039.

In contrast to the situation in the epithelial paradigms MDCK and Caco-2, the bulk of the GPI proteins was found on the basolateral surface. At the same time, epithelial lipid sorting was reversed. Compared with SM, newly synthesized C_6-NBD–GlcCer was preferentially delivered to the basolateral surface (van 't Hof W, Zurzolo C, Rodriguez-Boulan E, van Meer G, unpublished data).

49. •• DUPREE P, PARTON RG, RAPOSO G, KURZCHALIA TV, SIMONS K: **Caveolae and Sorting in the *Trans*-Golgi Network of Epithelial Cells.** *EMBO J* 1993, 12:1597–1605.

The authors identified VIP21, the canine homolog of caveolin, in transport vesicles from the *trans*-Golgi network in MDCK cells. The integral membrane protein is enriched in caveolae and in the *trans*-Golgi network. As it is relatively resistant to detergent extraction, like GPI proteins and glycosphingolipids [47••], a similar glycosphingolipid/GPI protein microdomain structure is proposed for caveolae as the putative apical precursor domain in the *trans*-Golgi network [43], and it is proposed that caveolin/VIP21 is involved in apical sorting.

50. •• HANNAN LA, LISANTI MP, RODRIGUEZ-BOULAN E, EDIDIN M: **Correctly Sorted Molecules of a GPI-Anchored Protein are Clustered and Immobile When They Arrive at the Apical Surface of MDCK Cells.** *J Cell Biol* 1993, 120:353–358.

The mobile fraction of a newly arrived GPI protein (gD1-DAF) at the plasma membrane was much less than that of its total plasma membrane population. In addition, clusters (of unknown size) were measured by fluorescence resonance energy transfer. Clustering but not immobilization also occurred in Con A-resistant MDCK cells that fail to sort gD1-DAF to the apical domain. Correct apical sorting is proposed to require pH-dependent association of the clusters to a transmembrane protein that interacts with the sorting machinery [43]. Some GPI proteins self-associate in a low pH- and ion-dependent manner [102•].

51. VAN MEER G: **Plasma Membrane Cholesterol Pools.** *Trends Biochem Sci* 1987, 12:375–376.

52. • DEVAUX PF: **Lipid Transmembrane Asymmetry and Flip-Flop in Biological Membranes and in Lipid Bilayers.** *Curr Opin Struct Biol* 1993, 3:489–494.

Highlights ideas on the mechanisms (proteins) responsible for phospholipid transmembrane segregation and scrambling.

53. SLOTTE JP, BIERMAN EL: **Depletion of Plasma-Membrane Sphingomyelin Rapidly Alters the Distribution of Cholesterol Between Plasma Membranes and Intracellular Cholesterol Pools in Cultured Fibroblasts.** *Biochem J* 1988, 250:653–658.

54. • MCINTOSH TJ, SIMON SA, NEEDHAM D, HUANG C-H: **Structure and Cohesive Properties of Sphingomyelin/Cholesterol Bilayers.** *Biochemistry* 1992, 31:2012–2020.

Differential scanning calorimetry, X-ray diffraction and measurements of cohesive properties by micromanipulation corroborate earlier evidence that the strong interaction between SM and cholesterol arises from the van der Waals interactions between cholesterol and saturated fatty acyl chains, and that there is no specific hydrogen bonding.

55. SCHROEDER F, NEMECZ G, WOOD WG, JOINER C, MORROT G, AYRAUT-JARRIER M, DEVAUX PF: **Transmembrane Distribution of Sterol in the Human Erythrocyte.** *Biochim Biophys Acta* 1991, 1066:183–192.

56. • KRISANS SK: **The Role of Peroxisomes in Cholesterol Metabolism.** *Am J Respir Cell Mol Biol* 1992, 7:358–364.

Summarizes the evidence for a functional cholesterol synthetic pathway in peroxisomes.

57. • DEGUCHI J, YAMAMOTO A, FUJIKI Y, UYAMA M, TSUKAHARA I, TASHIRO Y: **Localization of Nonspecific Lipid Transfer Protein (nsLTP = Sterol Carrier Protein 2) and Acyl-CoA Oxidase in Peroxisomes of Pigment Epithelial Cells of Rat Retina.** *J Histochem Cytochem* 1992, 40:403–410.

Electron microscopic colocalization of nsLTP with the peroxisomal marker anti-catalase.

58. • VAN HAREN L, TEERDS KJ, OSSDENDORP BC, VAN HEUSDEN GPH, ORLY J, STOCCO DM, WIRTZ KWA, ROMMERTS FFG: **Sterol Carrier Protein 2 (Non-Specific Lipid Transfer Protein) is Localized in Membranous Fractions of Leydig Cells and Sertoli Cells but Not in Germ Cells.** *Biochim Biophys Acta* 1992, 1124:288–296.

In addition, anti-sterol carrier protein 2 co-localized with anti-catalase on cryosections of Leydig cells with the EM immuno-gold technique.

59. URBANI L, SIMONI RD: **Cholesterol and Vesicular Stomatitis Virus G Protein Take Separate Routes from the Endoplasmic Reticulum to the Plasma Membrane.** *J Biol Chem* 1990, 265:1919–1923.

60. VANCE JE, AASMAN EJ, SZARKA R: **Brefeldin A Does Not Inhibit the Movement of Phosphatidylethanolamine from its Sites of Synthesis to the Cell Surface.** *J Biol Chem* 1991, 266:8241–8247.

61. • DAHL NK, REED KL, DAUNAIS MA, FAUST JR, LISCUM L: **Isolation and Characterization of Chinese Hamster Ovary Cells Defective in the Intracellular Metabolism of Low Density Lipoprotein-Derived Cholesterol.** *J Biol Chem* 1992, 267:4889–4896.

The mutant cell lines were defective in the regulatory responses elicited by LDL-derived cholesterol. The cells did respond to 25-hydroxycholesterol and mevalonate, showing that the defect was in the intracellular transport of the LDL-derived cholesterol, which accumulated in the lysosomes.

62. • SARRIA AJ, PANINI SR, EVANS RM: **A Functional Role for Vimentin Intermediate Filaments in the Metabolism of Lipoprotein-Derived Cholesterol in Human SW-13 Cells.** *J Biol Chem* 1992, 267:19455–19463.

In SW-13 adrenal tumor cell lines that lacked a vimentin network re-esterification of endocytosed LDL cholesteryl ester was 10-fold slower than in cells containing vimentin. Both cell types esterified endogenous cholesterol. The defect was overcome by transfecting the negative cells with a vimentin cDNA. No difference was observed in the apparent movement of cholesterol from the lysosomes to the plasma membrane.

63. • GOLDIN E, ROFF CF, MILLER SPF, RODRIGUEZ-LAFRASSE C, VANIER MT, BRADY RO, PENTCHEV PG: **Type C Niemann–Pick Disease: a Murine Model of the Lysosomal Cholesterol Lipidosis Accumulates Sphingosine and Sphinganine in Liver.** *Biochim Biophys Acta* 1992, 1127:303–311.

Hepatic levels of sphinganine and sphingosine were more than 20-fold higher in Niemann–Pick type C mice than in controls. The bases seem to accumulate in lipid-laden liposomes.

64. • BUTLER JD, BLANCHETTE-MACKIE J, GOLDIN E, O'NEILL RR, CARSTEA G, ROFF CF, PATTERSON MC, PATEL S, COMLY ME, COONEY A, *ET AL*: **Progesterone Blocks Cholesterol Translocation from Lysosomes.** *J Biol Chem* 1992, 267:23797–23805.

Progesterone blocked cholesterol esterification and led to lysosomal cholesterol accumulation.

65. • FELLERMANN K, REIMANN FM, HEROLD G, STANGE EF: **Mevinolin, a Competitive Inhibitor of Hydroxymethylglutaryl Coenzyme A Reductase, Suppresses Enterocyte Esterification of Exogenous but not Endogenous Cholesterol.** *Biochim Biophys Acta* 1992, 1165:78–83.

Mevinolin, at concentrations that did not reduce esterification of endogenously synthesized cholesterol, caused a nearly complete inhibition of esterification of LDL cholesterol in Caco-2 cells.

66. WATTENBERG BW, SILBERT DF: **Sterol Partitioning Among Intracellular Membranes. Testing a Model for Cellular Sterol Distribution.** *J Biol Chem* 1983, 258:2284–2289.

67. • DENNIS EA, VANCE DE: **Phospholipid Biosynthesis.** *Methods Enzymol* 1992, 209:1–544.

Collection of 63 papers reviewing our current knowledge on phospholipid biosynthetic enzymes.

68. VAN MEER G: **The Lipid Bilayer of the ER.** *Trends Biochem Sci* 1986, 11:194–195, 401.

69. •• BERR F, MEIER PJ, STIEGER B: **Evidence for the Presence of a Phosphatidylcholine Translocator in Isolated Rat Liver Canalicular Plasma Membrane Vesicles.** *J Biol Chem* 1993, 268:3976–3979.

Using water-soluble diC$_4$PC, which had been used to determine a PC-flippase activity in the ER [52•], evidence is presented for a similar pronase-sensitive activity in the bile canalicular but not the sinusoidal plasma membrane domain of rat liver cells.

70. •• BRATTON DL: **Release of Platelet Activation Factor from Activated Neutrophils. Transglutaminase-Dependent Enhancement of Transbilayer Movement Across the Plasma Membrane.** *J Biol Chem* 1993, 268:3364–3373.

Platelet-activating factor, 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine (PAF), is a short-chain PC that, when present on the cell surface, can be extracted by serum albumin. PAF release and uptake by the cell depended on cellular activation, and involved bulk transbilayer movement of plasma membrane phospholipids. Inhibition of this movement by monodansylcadaverine and methylamine also inhibited release and uptake of PAF.

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Newly synthesized PC was preferentially incorporated into transport vesicles generated from purified ER. CTP:phosphocholine cytidylyltransferase and 1,2-diacylglycerol:CDP-choline phosphotransferase remained associated with the vesicles and, upon phosphorylation, were released before fusion with Golgi [87•].

72. KAPLAN MR, SIMONI RD: **Intracellular Transport of Phosphatidylcholine to the Plasma Membrane.** *J Cell Biol* 1985, 101:441–445.

73. YAFFE MP, KENNEDY EP: **Intracellular Phospholipid Movement and the Role of Phospholipid Transfer Proteins in Animal Cells.** *Biochemistry* 1983, 22:1497–1507.

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In earlier work the group showed association of the PI transfer protein in mouse fibroblasts with the Golgi by immunofluorescence. Like Golgi markers, the protein was redistributed over the cytoplasm by BFA. Distinct labeling was also observed in the cytoplasm and nucleus. Association occurs when quiescent cells are stimulated by PMA or bombesin, which activate the phosphoinositide cycle. PMA (but not bombesin) increased phosphorylation of the PI transfer protein, possibly through protein kinase C. A regulatory function in Golgi transport similar to that of the structurally unrelated PI transfer protein in yeast is proposed.

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Reviews observations on agonist-responsive and -unresponsive pools of phosphoinositides. It is hypothesized that the activated receptor organizes a domain around itself including a phospholipase C. The portion of polyphosphoinositides that enters the domain is hydrolyzed.

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One of the most recent communications contributing evidence for the presence of a complete phosphoinositide cycle in the nucleus that is functional *in vivo*, and referring to the most relevant earlier work.

79. TING AE, PAGANO RE: **Detection of a Phosphatidylinositol-Specific Phospholipase C at the Surface of Swiss 3T3 Cells and its Potential Role in the Regulation of Cell Growth.** *J Biol Chem* 1990, 265:5337–5340.

80. • GNAMUSCH E, KALAUS C, HRASTNIK C, PALTAUF F, DAUM G: **Transport of Phospholipids Between Subcellular Membranes of Wild-Type Yeast Cells and of the Phosphatidylinositol Transfer Protein-Deficient Strain *Saccharomyces cerevisiae* Sec 14.** *Biochim Biophys Acta* 1992, 1111:120–126.

Both earlier work and the present paper offer a wide range of observations. Transport of PC and PI to the plasma membrane was not slowed down (but faster) when the yeast PI/PC transfer protein (sec14) was inactive (and vesicular glycoprotein transport was blocked). Transport was much faster than in control cells, which is difficult to understand.

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83. KOBAYASHI T, ARAKAWA Y: **Transport of Exogenous Fluorescent Phosphatidylserine Analogue to the Golgi Apparatus in Cultured Fibroblasts.** *J Cell Biol* 1991, 113:235–244.

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In a previous report (pp 5686–5692) the group localized activated membrane-bound cytidylyltransferase to the nuclear membrane by immunofluorescence. Now by the same method, the soluble, inactive form of the enzyme has benn localized to the nuclear matrix (but see [71•]).

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Ca^{2+} favored the transfer of newly synthesized PS in a cell-free system as monitored by decarboxylation. No other soluble factors were required. Transferred PS had a higher specific radioactivity than microsomal PS, suggesting subcompartmentalization of PS synthesis.

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90. •• VOELKER DR: **The ATP-Dependent Translocation of Phosphatidylserine to the Mitochondria is a Process that is Restricted to the Autologous Organelle.** *J Biol Chem* 1993, 268:7069–7074.

PS transport to mitochondria as monitored by decarboxylation was reconstituted in a cell-free system and required ATP and the Ca^{2+}-sequestering ATPase. When Chinese hamster ovary cells with hydroxylamine-inactivated decarboxylase were homogenized, PS, radiolabeled in the homogenate, was not converted to PE. Surprisingly, PS was not decarboxylated when untreated mitochondria with active decarboxylase and ATP were added either. With the previous observation that PS transport to the mitochondria was insensitive to dilution, a physi-

cal (ATP-dependent?) association of a specialized domain of the ER with the mitochondria is favored.

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Radiolabeled PS inserted into isolated mitochondria by means of a transfer protein was rapidly decarboxylated. Both labeled PS and PE were confined to the outer membrane. The decarboxylase was not found in contact site fractions. Experiments using 1,4-dinitrophenol suggest the involvement of contact sites in transport between inner and outer membrane. PS-derived PE did not mix with pre-existing inner membrane PE.

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PS and PE analogs with a pyrene-labeled acyl chain, inserted into isolated mitochondria by a transfer protein, were not found in the inner membrane. Similar behavior of the PS and PE analogs argued against an enrichment of PS at contact sites. Decarboxylation was independent of changes in PS acyl chain length, suggesting that transport involved lateral diffusion rather than monomeric exchange.

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