Metabolism and Translocation of Aminophospholipids In Mammalian Cells

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Academic dissertation

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ORIGINAL PUBLICATIONS

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III. Kainu V, Hermansson M, Hänninen S, Hokynar K and Somerharju P. (2011): **Import of phosphatidylserine to and export of phosphatidylethanolamine molecular species from mitochondria.** *Manuscript submitted for publication.*

ABBREVIATIONS

AA	arachidonic acid
BEL	bromoenollactone
BHK cells	baby hamster kidney cells
CHO cells	Chinese hamster ovary cells
CL	cardiolipin
ER	endoplasmic reticulum
ESI-MS	electrospray ionization masspectrometry
HA	hydroxylamine
HF cells	human fibroblast cells
HPLC	high performance liquid chromatography
IMM	inner mitochondrial membrane
LTP	lipid transfer protein
MAFP	methyl arachidonyl fluorophosphonate
MAM	mitochondria- associated membrane
Meβ-CD	methyl-β cyclodextrin
NL	neutral loss
OMM	outer mitochondrial membrane
PA	phosphatidic acid
PC	phosphatidylcholine
PE	phophatidylethanolamine
Pemt	phosphatidylethanolamine N-methyltransferase
PG	phosphatidylglycerol
PI	phosphatidylinositol
PLA	phospholipase A
PM	plasma membrane
PS	phosphatidylserine
PSD	phosphatidylserine decarboxylase
SM	sphingomyelin

ABSTRACT

In this study we investigated the metabolism, i.e. remodelling and translocation, of the aminophospholipids phosphatidylserine (PS) and phosphatidylethanolamine (PE). A new method for introduction of exogenous PS and PE molecular species to cultured cells was developed, and combined with mass spectrometry it enabled more detailed follow-up of the metabolism of single molecular species than was possible previously.

We found that I) exogenous PS and PE molecular species can be efficiently introduced to cultured cells without compromising cell integrity, II) PS and PE molecular species are remodelled by several phospholipases displaying selectivity based on phopholipid head group and acyl chain composition, III) PS decarboxylase (PSD) and the Kennedy pathways provide a different PE molecular species composition to the cellular PE pool. In addition, PE species produced by these pathways are translocated from the site of synthesis to other cell compartments depending on their acyl chain composition.

The data obtained in the present study helps to understand cellular phospholipid metabolism in more depth. The data show that effective labelling of cultured cells by exogenous phospholipids does not compromise cell viability and may be used to disturb cellular phospholipid composition to study lipid homeostasis. Remodelling and translocation of PS and PE molecular species is highly selective. The developed method and mass- spectrometric techniques may be used in future studies to understand disturbances in lipid homeostasis for example in diabetes mellitus, thus opening doors to optional scientific approaches for studying the mechanisms behind pathologies related to lipid disturbances.

INTRODUCTION

Mammalian cells and subcellular organelles are enclosed by membranes consisting of hundreds of different lipid species and proteins. Lipids also store energy, regulate the activity of some proteins and serve as second messengers in signal transduction (or as sources of such messengers), e.g. the platelet activating factor and eicosanoids [1, 2].

It has been estimated that mammalian cell membranes contain over 1000 different types of lipid molecules differing in the head group and acyl chains [3]. Phospholipids define the fluidity of the membrane, regulate its properties as well as the properties of embedded proteins [3]. Phospholipids are also present in heterogeneities referred to as lipid rafts [4, 5].

The most abundant phospholipid in mammalian cells is phosphatidylcholine, comprising 40-50% of the total phospholipids [3]. Phosphatidylethanolamine and phosphatidylserine comprise 15-25% and 2-10% of the total cellular phospholipids while the other phospholipid classes, phophatidylinositol, sphingomyelin, cardiolipin, phosphatidic acid and glycosphingolipids comprise 10-15%, 5-10%, 2-5%, 1-2% and 2-5% respectively [3]. The properties of the different phospholipids, for example those of the head groups influence membranes in diverse ways. Phosphatidylethanolamine in phosphatidylcholine bilayers induces curvature stress to the membrane thus enabling the fission, fusion and budding of membranes [6]. In addition phosphatidylethanolamine and cardiolipin participate in accommodation and modulation of membrane proteins and their activities [6, 7].

The membrane phospholipid composition is strictly controlled both at cellular and tissue levels [8-10]. For example, the brain tissue and the retina are enriched in phosphatidylethanolamine and phosphatidylserine [11]. Another example is the enrichment of arachidonic acid (20:4) in the phospholipids of pancreatic islets [12]. In addition, the phospholipid acyl chain composition is tailored for the purpose of the specific tissue, as in the grey matter where 36% of the fatty acids in phosphatidylserine is docosahexaenoic acid (22:6) [3, 12, 13]. This enrichment in 22:6 fatty acid has been proposed to be vital for normal development and function of the nervous system [3, 13].

In humans, alterations in lipids are involved in various diseases or pathological processes, such as atherosclerosis and type 2 diabetes. Recent advances in lipidomic methods [14] should help to understand the role lipids play in these common diseases and may thus allow one in future the design of preventive actions.

This work focuses on the metabolism and translocation in mammalian cells of the aminophospholipids phosphatidylserine (PS) and phosphatidylethanolamine (PE), which were identified decades ago [3, 7], but whose biological functions are still poorly understood.

LITERATURE REVIEW

1. Mammalian Glycerophospholipids

1.1. Structures and organellar distribution

1.1.1. General structures

The glycerophospholipids are composed of a polar head group, a phosphate group, a glycerol moiety and acyl chains. The phospholipid classes are delineated by the polar head group, while the different molecular species are distinguished by their acyl chain compositions (Fig. 1).

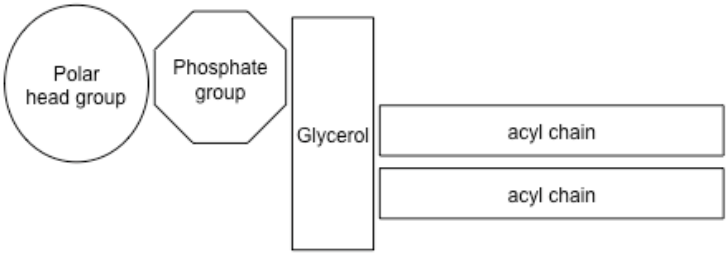

Figure 1. *Simplified model of the glycerophospholipid structure* – In principle the glycerol and phosphate groups are universal building blocks, whereas the polar head group defines the PL class and the acyl chains the PL molecular species.

1.1.2. Molecular species diversity

The phospholipid (PL) molecular species vary in acyl chain length and/or unsaturation. Typically, the *sn1* position contains a saturated or monounsaturated acyl chain and the *sn2* position a mono- or polyunsaturated acyl chain [10, 15]. The *sn2* acyl chain is ester-linked, while that in the *sn1* position may be ester-, ether- or vinylether-linked to the glycerol moiety [16]. The acyl chains are commonly 14- 24 carbons long and contain between 0- 6 double bonds [16].

1.1.3. Organelle phospholipid composition

Each subcellular organelle has a distinct protein and lipid composition [7]. The plasma membrane (PM) is enriched in sphingolipids and sterols [17, 18], while the endoplasmic reticulum (ER) contains only low levels of these lipids [17, 18]. The early endosomes resemble the PM in their lipid composition, but during their conversion to lysosomes their PS and sterol contents decrease while the lysobisphosphatidic acid content increases [19, 20]. The lysosomes resemble the late endosomes with a lower PS content compared to the PM [3].

Mitochondria contain only small amounts of PS and trace amounts of sterols and sphingolipids [21], while they are enriched in PE. In the rat liver the PE constitutes 20% of the ER phospholipids whereas it constitutes 39% and 35% of the inner and outer mitochondrial membrane phospholipids respectively [22]. Mitochondria contain 40% of PC, 30% of PE, 10-15% of CL and 5% of PI, PA and PS [22, 23]. Mitochondria also contain CL and PG that are not found in other cell organelles [3, 24]. The CL is enriched in the inner mitochondrial membrane [3]. Mitochondrial phospholipids also display a distinct

acyl chain composition with relatively more of unsaturated (polyunsaturated) acyl chains than in other subcellular organelles [24].

1.2. Membrane phospholipid asymmetry

1.2.1. Plasma membrane

The plasma membrane is highly asymmetric. Up to 80% of PE and essentially all of PS is located on the cytoplasmic leaflet while most of the PC and SM is in the exoplasmic leaflet [7, 25]. This asymmetry is particularly well established in erythrocytes [25]. The bilayer asymmetry relates to biological functions such as the various effects of PS externalization, the membrane curvature affecting properties of PE and the interactions between plasma membrane embedded proteins with PC, SM and sterols. The asymmetry may also contribute to for example cell structure and cell-cell interactions [26, 27].

1.2.2. Endoplasmic reticulum

Phospholipid biosynthesis is mainly conducted on the cytosolic side of the ER thus resulting in the necessity for transbilayer movement of the PLs that stay in the ER to maintain leaflet homeostasis [28]. For example PE is synthesized on the cytosolic side of the ER and it is rapidly transported to the luminal side, with a half time of 20 min [29]. Also, PC and lysophosphatidylcholine are transported. The translocation of these PLs is significantly attenuated by protease treatment, but it is not affected by addition of ATP [30-32], however, a distinct transporter has not been identified.

1.2.3. Flippases, floppases and scramblases

Spontaneous transbilayer movement of phospholipids in model membranes is slow. However, in cell membranes this movement has been estimated to occur very rapidly, with half times in the order of seconds or minutes [33-35]. The rapid transbilayer movement is evidently due to active transbilayer transport [36].

The transport of PS from the exoplasmic leaflet to the cytoplasmic leaflet of PM is mediated by an ATP-dependent aminophospholipid translocase referred to as flippase. However, the protein involved remains unidentified [7]. The activity of this flippase varies between cell types. Human fibroblasts have a one order of magnitude higher aminophospholipid translocase activity than erythrocytes [37]. P-type ATPases have been suggested to be responsible for the flippase activity [36, 38]. PS and PE are preferred substrates for the ATP-dependent flippases, but PC has also been shown to be transported by such proteins [39].

Flippase activity has been found in e.g. plasma membrane of erythrocytes [34, 38], platelets [38], fibroblasts [40, 41], spermatozoa [42], epithelial cells [39] and hepatocytes [43]. Flippase activity has also been detected in subcellular organelles such as the Golgi apparatus [44]. Flippase activity is ATP-dependent and sensitive to vanadate and cellular calcium [38, 45]. The transport of spin-labelled PS was found to be 10- fold faster than that of PE [34, 38, 46]. Flippase does not discriminate between serine enantiomers or between natural PS molecular species [26, 38, 47].

Lipid transport from the cytoplasmic side to the exoplasmic side of PM is conducted by proteins referred to as floppases. The transport is distinct from the flippase activity [48]. The floppases seem

less specific for the polar head group than the flippases [48]. Floppase activity has been ascribed to be (partly) mediated by P-glycoproteins that transport amphiphilic drugs and phospholipids via an ATP dependent mechanism [49, 50].

The family of ATP-binding cassette (ABC) proteins has been associated with transbilayer transport of phospholipids by functioning as floppases [51]. ABCA1 was suggested to play a key role in apoptotic events by promoting transient and local surface exposure of PS in apoptotic cells [52, 53]. However, this role has been questioned since there is major scrambling of all phospholipids and not just PS during apoptosis [54, 55]. ABCA1 is expressed ubiquitously in human cells [56]. In humans, mutation in the ABCA1 protein results in Tangier's disease [57, 58]. ABCB4 in turn has been located to the bile canalicular membranes where it transports PC to the bile [3, 46]. In humans dysfunction of ABCB4 may result in hepatic cholestasis, while mutation in ABCA4, which is located in the rod and cone photoreceptors where it transports retinoids, causes Stargardt's macular dystrophy [59, 60].

Scramblases move phospholipids bidirectionally in the PM and thereby "scramble" the phospholipid asymmetry [7]. Scramblases have been proposed to be enriched in the so-called lipid rafts [61, 62]. The scramblase activity was found in erythrocytes displaying transbilayer movement of phospholipids that was ATP-independent and required increased intracellular calcium levels [63]. Scramblases have somewhat higher efficiency towards glycerophospholipids than sphingolipids. A protein expressing scramblase activity has been purified from human erythrocytes and platelets [64, 65]. However, the specific protein that causes the PM phospholipid scrambling has not been identified. Members of the scramblase family are found also in the intracellular membranes and the deletion of phospholipid scramblase 1 gene in mice did not affect lipid transport [63], thus questioning the role of scramblase proteins. Indeed it has been suggested that the increase in Ca^{2+} concentration itself could promote the surface exposure of PS and PM phospholipid scrambling [66].

1.3. Interorganelle trafficking

Interorganelle phospholipid translocation is required for distribution of PLs from ER (site of synthesis) to other organelles. Lipids do not have intrinsic structures that would direct them to their destination, yet the subcellular organelles have distinct membrane lipid compositions [67, 68]. Lipids have a low solubility in the aqueous phase suggesting the presence of a distinct transport mechanism since simple spontaneous diffusion would be too slow to fulfill the cell's requirements [69, 70]. Membrane lipid compositions and their biophysical properties could further affect the kinetics of lipid transfer, to help appropriately direct the lipid transport [71, 72].

1.3.1. Vesicular transport

Vesicles transporting proteins inside the cell consist of phospholipids. Accordingly, vesicular transport of lipids must occur on the routes of protein transport between the Golgi, PM and the ER [63]. Also, the endocytotic pathways could participate in lipid translocation from the PM to the subcellular organelles [73, 74].

Indeed, nocodazole (a microtubule destabilizing drug) inhibited the transport of fluorescent PC and caused accumulation of PC in the endosomes [75]. Also the transport of nascent sphingomyelin from the Golgi to the plasma membrane was inhibited by addition of monensin which inhibits protein traffic through the Golgi [76]. In addition, arresting vesicle traffic in mitotic cells inhibits the transport of sphingomyelin to the PM [77].

1.3.2. Protein-mediated transfer

Arresting vesicle transport by depletion of cellular ATP or reduction of temperature does not abrogate the transport of phospholipids from the ER to PM [78-80]. Studies with isolated organelles and permeabilized cells have shown that transport of phospholipids occurs even in the absence of the cytosolic factors, ATP and GTP [81-83]. Lipid transport also occurs between organelles that do not participate in the vesicular pathways including peroxisomes and mitochondria [84, 85]. Non-vesicular transport has been shown for PC, PE and PS between ER, mitochondria, the Golgi and the PM [78-80, 83].

The non-specific lipid transfer protein (nsLTP) has been isolated from the cytosol of human, rat, bovine and goat liver and *in vitro* it has been shown to display broad substrate specificity. The protein has been localized to the cytosol and peroxisomes [86, 87]. In mutated CHO cells lacking this specific protein the synthesis, transport and decarboxylation of PS was unaltered as compared to normal cells [88].

A protein transporting PI and PC was isolated from bovine brain where it was localized to the Golgi membranes [87]. The PI transfer protein has been suggested to be an important component in phosphoinositide signal transduction by transporting PI for phosphorylation in the Golgi/PM [89].

Various proteins have been described to mediate lipid exchange between membrane populations *in vitro* [71, 90]. However, solid evidence for the role of such proteins *in vivo* is still lacking. It has been suggested that the proteins rather might facilitate lipid transfer between membranes and locally modulate the lipid composition, thereby regulating vesicle trafficking, signal transduction and lipid metabolism [71, 91]. The lipid transfer proteins have been suggested to participate in lipid transfer at membrane contact sites, for example the ceramide transfer protein (CERT) that transports ceramide at the ER and Golgi contact sites [92, 93].

1.3.3. Spontaneous diffusion

Several studies have provided evidence for spontaneous movement of lipid monomers, such as the transport of newly synthesized PC from its site of synthesis to the PM, which is independent of ATP as well as insensitive to disruption of protein traffic through the Golgi (by monensin) and cytoskeletal poisons (colchicines and nocodazole) [94-96].

The transport to the PM is also very rapid [78]. The rate of spontaneous movement of PLs between membrane compartments is proportional to the lipid's solubility in aqueous-phase which depends on acyl chain length and saturation [95, 97]. Kinetic measurements suggest two types of spontaneous translocation [71]. The first type is aqueous diffusion occuring at low membrane concentrations and the determining factor is the concentration of the donor membranes, the rate-limiting step being the desorption of the lipid from the donor bilayer [71, 95]. The other mechanism depends on membrane collisions and that the lipid to be translocated partially extends from the donor membrane thereby increasing the propability of transfer when the donor and acceptor membranes collide [98]. This mechanism requires high concentrations of both donor and acceptor membranes [69].

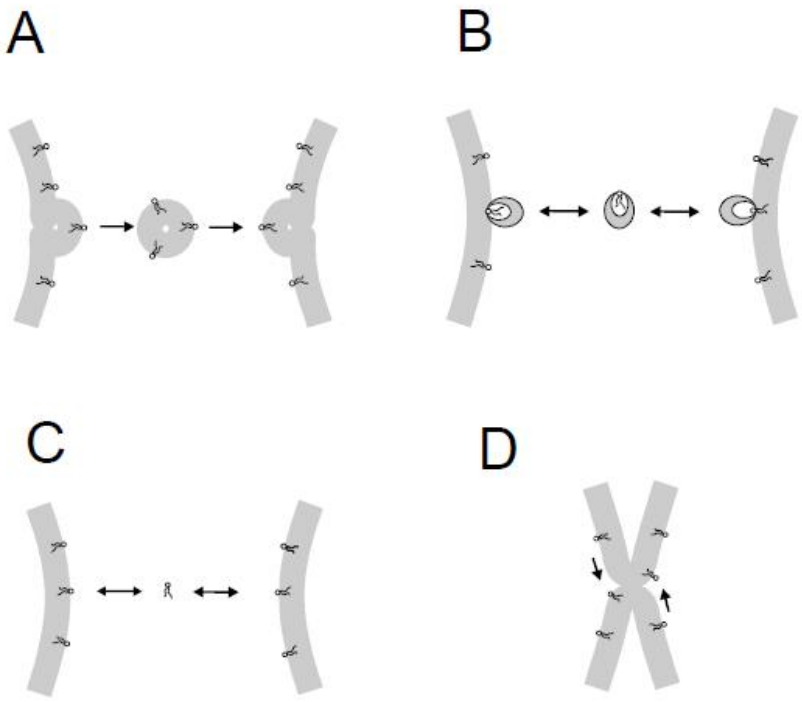

Figure 2. *Mechanisms of phospholipid translocation* – PLs may be translocated alternatively via vesicles used e.g. for protein transport (A), by phospholipid transport proteins (B), by spontaneous diffusion (C) or via organelle contact sites such as those between the MAM and mitochondria (D).

1.3.4. Contact site –dependent transfer

Phospholipid translocation has been suggested to occur at site of membrane contacts [93, 99]. The lipids may by lateral diffusion be transported between membranes at membrane contact sites between for example the PM and lipid droplets, ER and mitochondria and lipid droplets and mitochondria [100, 101]. A membrane contact site is defined as a site with a very narrow gap (10 - 20nm) between the membranes of two organelles enabling traffic of calcium, metabolites and lipids by non-vesicular mechanisms [85, 102]. The contact sites are formed and/or stabilized by connection to the apposing membrane by protein-protein or protein-lipid interactions. Several proteins implicated in contact site formation and stabilization have been identified [103-106]. Examples are the voltage-dependent anion channel 1 (VDAC1) that may interact with several ER and mitochondrial proteins [107, 108], and STIM1/Orai which functions as an ER Ca^{2+}- sensor [109]. Membrane contact sites may result in hemifusion of the apposing membranes and not only enhance their proximities (Fig. 2).

An example of membrane contact sites playing a role in phospholipid transport are those between mitochondria and the ER/MAM. MAM have been shown to be in close contact with mitochondria and it has been suggested that they mediate the transport of PS [110-112]. The role of MAM in the lipid transport has been studied in mnd/mnd mice which have decreased number of ER- mitochondria contacts. However, their mitochondrial phospholipid composition was found to be normal [113].

The transport of ceramide between the ER and the Golgi has also been proposed to occur at membrane contact sites [114, 115]. In fact the ceramide transfer protein (CERT) has been shown to link the ER and the Golgi by binding to the ER and Golgi membranes by the protein's FFAT motif and PH domain [92, 93]. In yeast an ER-mitochondria tethering complex called ERMES has been implicated to have a role in determining the mitochondrial CL and PE composition [116]. However, cells with

mutated ERMES proteins do not display total inhibition of PL translocation to mitochondria, thus suggesting that translocation via ER-mitochondria contact sites is not the only pathway for PL transport to mitochondria in yeast [116]. Homologues of ERMES proteins have not been found in mammalian cells. However, two proteins, Mitofusin 2 and Mitostatin, have been implicated to participate in MAM/mitochondria contact sites [104, 117].

2. Aminophospholipids and their functions

2.1. Signal transduction

Phospholipids have many distinct roles in organisms including intra- and intercellular signalling. Some phospholipids serve as precursors for lipid second messengers such as diacylglycerol, inositol-1,4,5 trisphosphate [7, 118], lysobisphosphatidic acid and arachidonic acid [7], or they can directly affect proteins with signalling functions. For example PS has been reported to function as a cofactor or activator of protein kinase C, which plays an important role in cellular signalling [119-122]. In addition, PS is thought to direct certain proteins to cell membranes [123].

PE which tends to form cubic phases *in vitro* [124] affects cellular signalling via its physical properties and as a precursor of second messengers. In the PM, PE modulates the membrane curvature and thus has a role in membrane fission and fusion [7]. In addition, PE is required at the cleavage furrow in the contractile ring assembly during cytokinesis [7]. The ethanolamine moiety from PE is used in glycosylphosphatidylinositol anchors of several cell surface signalling proteins [7]. PE also serves as a precursor for anandamide [125].

2.2. Apoptosis

The transbilayer distribution of PS in the PM is connected to its role in apoptosis. PS is externalized during the early phases of apoptosis, thus probably serving as a signal for phagocytes [126-128]. It has been suggested that macrophages possess a PS binding receptor, but it remains to be identified [129-132]. PS may be externalized by the putative scramblase (cf. 1.2.3) [133].

The PS molecules that are exposed at the cell surface originate from a pool of newly synthesized PS, which may be synthesized by PS synthase 1 or PS synthase 2 [134-136]. It has been shown that neither PS synthase is individually responsible for providing the pool of PS that is externalized during apoptosis [7, 137]. In addition, a twofold increase in PS synthesis during apoptosis has been reported [136, 138].

2.3. Blood clotting

PS is also tightly connected to blood-clotting, since the exposure of PS at the surface of platelets is thought to initiate this event [139, 140]. The externalized PS interacts with proteins of the coagulation system [141], leading to an increase in thrombin, which causes conversion of fibrinogen to fibrin and subsequently platelet aggregation [139, 142, 143]. High local concentrations of PS are needed for the proteolytic activity of the complex consisting of factor VIIa and tissue factor [144]. In addition, conversion of prothrombin to thrombin is stimulated by PS and coagulation factor Xa [145].

2.4. Sperm maturation

Externalization of PS has been reported to be part of sperm maturation. It has been proposed to be due to decreased inward movement in capacitated cells [146, 147]. The externalization of PS could be mediated by an ATP-dependent transporter expressed only in the acrosomal region of sperm [3, 148]. This transporter has also been found to be crucial for the normal binding and penetration of the zona pellucida of the egg cell.

2.5. PS surface exposure under pathologic conditions

PS externalization has been connected to several hematologic pathologies. In Scott syndrome, the platelets function normally, but have impaired scramblase activity, leading to impaired externalization of PS [149]. In antiphospholipid syndrome circulating antiphospholipid antibodies seem to target plasma proteins interacting especially with PS resulting in e.g. arterial and venous thrombosis [150-152]. In sickle cell anemia, a subpopulation of cells is lacking detectable aminophospholipid translocase activity and displays a partial collapse of membrane phospholipid asymmetry including surface exposure of PS [153-155]. In thalassemia, which causes congenital hemolytic anemia, the complications might be due to the procoagulant effect of a PS-exposing subpopulation of erythrocytes [152, 156].

In approximately 3% of uremic patients a subpopulation of erythrocytes express PS at their surfaces [157]. Uremic patients undergoing repeated peritoneal dialysis have less erythrocytes expressing PS and less severe anaemia [152, 157]. The exposure of PS is also connected to kidney stone disease [158, 159]. In diabetes mellitus erythrocytes and platelets show partial collapse of membrane phospholipid asymmetry as well as exposure of PS [152, 160].

Infected cells express PS when going to apoptosis and it has been found that several virus-infected cells exhibit a procoagulant phenotype [152, 161]. Also bacterial infections cause exposure of PS in the cells, an example of such bacteria is *Chlamydia pneumonie* that has also been found in atherosclerotic lesions [152, 162]. A significant increase in PS exposure has also been reported in tumorigenic vs. nontumorigenic cells [152, 163, 164].

3. Metabolism of Aminophospholipids

3.1. Phosphatidylserine

3.1.1. Biosynthesis

Mammalian cells contain two known PS synthetases, PSS1 and PSS2, which use PC or PE, respectively, as substrates in a base-exchange reaction (Fig. 3) [165]. It has been proposed that there is a third an ER-specific enzyme that would explain the PS synthesis activity in ER [3, 135]. PS synthesis occurs in the ER with the highest specific activity in MAM [112, 135, 166]. The synthesis itself is not dependent on energy but a sufficient Ca^{2+} concentration is required [7, 83, 167].

In vitro, PSS1 utilizes both PC and PE as substrates [3]. The PE and PC consumed in the reaction can be replenished by the Kennedy pathway, which can incorporate the released choline or ethanolamine back to PC and PE [168]. The two PSS proteins are predicted to have several membrane spanning domains and the amino acid sequences are 30% identical [165, 169, 170].

The two PS synthases are encoded by different genes [171, 172]. The PSS1 gene has been localized to chromosome 13 in the mouse and to chromosome 8 in humans [3, 173]. The relative expression levels of the two PS synthases vary between tissues suggesting that they could have specific functions [7]. The PSS1 mRNA is expressed ubiquitously in mouse tissues and especially abundant in liver, kidney and brain tissues. The PSS2 mRNA is mostly expressed in Sertoli cells of the testis and at significantly lower levels in the heart and brain. In the brain PSS2 is expressed especially in the Purkinje cells of the cerebellum and the pyramidal neurons of the hippocampus [173-175].

Mice models deficient in either PSS1 or PSS2 have been generated [7, 174]. The PSS1 knock-out mice showed no obvious phenotype and both genders were fertile [7]. The PSS2 $^{-/-}$ females were fertile, but the males displayed smaller testes than normal and approximately 10% of them were infertile with atrophied testes and spermatic ducts lacking spermatocytes, hyperplasia of Leydig cells and an abnormally thick layer of Sertoli cells [174]. In contrast to the single knock-out mice the double knock-out are not viable [176].

3.1.2. Regulation of PS synthesis

The regulation of PS synthesis is a key issue in cellular PS homeostasis. The synthesis is regulated by the cellular PS level [177]. However, only little is known about the transcriptional regulatory mechanisms of the expression of the PSS genes [7].

The contribution of the two PSS enzymes to PS synthesis was studied using CHO cells deficient in PSS1. These cells displayed a 35-55% lower PS-synthesis than normal cells and decreased amounts of PS and PE [171, 172]. In addition, their growth was severely impaired if not supplemented with PS, PE or ethanolamine [171, 172]. The PS synthesis activity was lowered by 95% as compared to parental cells when PSS1 deficient CHO cells were further mutagenized resulting in an 80% decrease of PSS2 mRNA. These cells were viable only when supplemented with PS [170, 178]. Overexpression of PSS2 eliminated the need for PS supplementation in PSS1 deficient CHO cells [175] indicating that there is no intrinsic need for PSS1 in CHO cells [7].

In rat brain, phosphorylation of PSS1 was suggested to regulate the synthesis of PS [179]. There are also implications that the synthesis is regulated by the need for distinct PS species since synthesis by PSS1 in brain and liver microsomes prefers PC species containing docosahexaenoic acid as substrates [13]. In CHO cells, increased cellular PS level resulted in reduced rates of incorporation of radiolabel into PS [177]. In addition, the activity of PSS2 purified to near homogeneity, was inhibited by addition of PS but not PE or PC [180] , indicating that PSS2 is inhibited by direct interaction with PS [180, 181]. Inhibition of PS synthesis by exogenous PS was absent in a CHO mutant (mutant 29) [182]. It was found that a point mutation in the PSS1 gene was behind the aberrant regulation of PSS activity [183]. In this mutation Arg-95 is changed to lysine causing the resistance to end product inhibition of the synthesis. Similarly, in PSS2 an arginine residue is located at position 97 and changing this residue to lysine results in a 4- fold higher PS synthesis compared to parental CHO-K1 cells [181].

When PSS1 was overexpressed in McArdle 7777 rat hepatoma cells, incorporation of ^{3}H-serine into PS was increased 3-fold, but the cellular levels of PS or PE did not increase [184], possibly due to an increase in the PSD pathway and a decrease in the CDP-ethanolamine pathway of PE synthesis [184]. In contrast, the equivalent overexpression of PSS2 did not increase the incorporation of ^{3}H-serine into PS [175]. However, overexpression of PSS2 in CHO cells increased the ethanolamine-exchange activity by 10-fold, whereas the choline-exchange activity was unaffected as expected [178] indicating

that PSS2 is responsible for the ethanolamine exchange activity. These data implicate tight regulation of PS synthesis activity in order to maintain optimum levels of PS [7].

PSS2 $^{-/-}$ mice do not have severe developmental abnormalities or changes in PL composition [174].The hepatocytes of PSS2 $^{-/-}$ mice had normal levels of PS and PE, possibly due to increased PSS1 activity and decreased PS degradation, but not due to increased PSS1 mRNA levels [185].

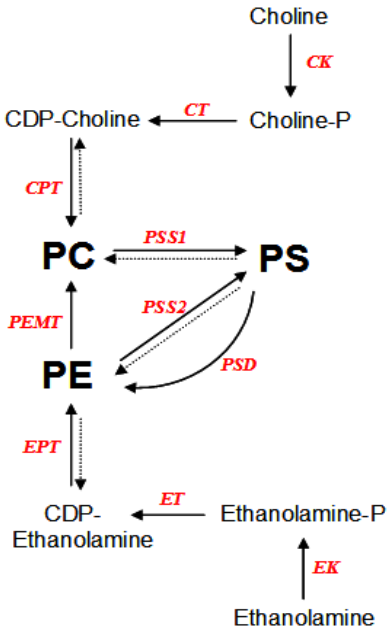

Figure 3. *Synthesis of PS and PE* – PS is synthesized by a base-exchange reaction with the highest activity in the MAM by either PS synthase 1 or PS synthase 2. PE on the other hand may be synthesized in ER/MAM *de novo* by the Kennedy pathway, which is similar to that of PC, or via decarboxylation of PS, by PS decarboxylase (PSD) in the mitochondria.

3.2. Phosphatidylethanolamine

3.2.1. Biosynthesis

PE is synthesized via two alternative pathways, the CDP-ethanolamine (Kennedy) pathway [186] and the PS decarboxylation pathway (Fig. 3) [168]. In mammals the precursor, ethanolamine, is mainly obtained from the diet or from degradation of PE derived from PS. In addition small amounts of ethanolamine are generated upon degradation of sphingolipids [187].

The first reaction in the Kennedy pathway is catalyzed by ethanolamine kinase [188]. Two different isoforms of this enzyme exist. One, EK2, is able to phosphorylate both ethanolamine and choline, whereas another, EKI1 can only phosphorylate ethanolamine [188, 189]. The second step, often considered as the rate-limiting one, is catalyzed by CTP-ethanolamine cytidylyltransferase. The third and final step, i.e. reaction of CDP- EA with diacylglycerol, is catalalyzed by the CDP-ethanolamine diacylglycerol ethanolaminephosphotransferase [190, 191].

Only one gene encoding CTP-ethanolamine cytidylyltransferase has been identified in eukaryotes [7]. The mRNA encoding choline/ethanolamine phosphotransferase has been shown to be ubiquitously

present in different human tissues. The enzymatic activity is found primarily in the ER [166, 190, 192].

The PS decarboxylase, first discovered in bacteria [193], contributes to the majority (up to 80%) of the PE pool even when ethanolamine is included in the cell culture media in BHK and CHO cells [168, 194, 195], while in rat hepatocytes and hamster heart most of the PE is derived from the Kennedy pathway [7, 196]. In CHO cells, the CDP-ethanolamine pathway mainly produces molecules with mono- or diunsaturated acyl chains at the *sn2* position, whereas the PSD pathway preferentially produces species with polyunsaturated acyl chains at this position [197]. PSD may act selectively on PS species with different acyl chains, however this has not been convincingly proved [197-200].

In mammalian cells PS decarboxylase is found only in the inner mitochondrial membrane [201, 202] with its active site facing the intermembrane space [7, 200, 202]. The cDNA encoding the PS decarboxylase was identified in complementation experiments with CHO cells defective in PS decarboxylase activity [203]. The PSD protein consists of 409 amino acids [204] and the amino terminus of the protein contains regions that target proteins to the mitochondria which thus explains its localization [204, 205]. The complete processing of the PS decarboxylase in mammalian cells is shown to occur in three proteolytic steps completed during localization to mitochondria [200, 204].

3.2.2. Regulation of PE synthesis

The regulation of the CDP-ethanolamine pathway is not known well. The major regulatory step is presumably the reaction catalyzed by the CTP-ethanolamine cytidylyltransferase [196, 206], albeit under certain conditions, the preceeding step, catalyzed by the ethanolamine kinase, may be rate-limiting [188]. Mice lacking the ethanolamine-specific kinase have increased perinatal mortality, but have normal liver PE content [207], probably because choline/ethanolamine kinase can compensate for the defect [7].

The reason for the existence of the PSD pathway in mammals is not well understood, but it could be necessary to provide adequate amounts of PE to the mitochondrial membranes [208, 209]. PS subjected to decarboxylation has been suggested to originate from a pool of newly synthesized PS [81, 82, 112] and there is evidence that the PE formed by decarboxylation does not readily mix with the PE already present in the inner mitochondrial membrane [210]. PSD deficient mice do not survive past the ninth embryonic day. This strongly suggests that the PSD pathway is essential for development [209]. Fibroblasts of the PSD $^{-/-}$ mice display fragmented and aberrantly shaped mitochondria implicating that the reduced mitochondrial PE lies behind the observed defects and the embryonic lethality. The PSD $^{+/-}$ mice, on the contrary, appear normal and display normal mitochondria and PE content in the liver and other tissues. In the heterozygous mice the activity and amount of the CTP-phosphoethanolamine cytidylyltransferase was found to be increased implicating increased synthesis of PE via this pathway possibly compensating for the PSD deficiency [209].

Both pathways for synthesis of PE, i.e. the PSD and the CDP-ethanolamine pathways, seem to be essential for normal mouse development since also Pcyt2 $^{-/-}$ (the gene encoding the CTP-phosphoethanolamine cytidylyltransferase), mice are embryonic lethal in addition to PSD $^{-/-}$ mice [7].

3.3. Remodelling of (PS and PE) phospholipids

Theoretically, all phospholipid molecules present in the cell could be synthesized directly *de novo*. However, in most cells many of the species are formed via remodelling, i.e. one or both of the acyl chains are exchanged for others [211]. The *sn2* position is more often remodelled than the *sn1* one [212]. The remodelling requires a sequential action of A-type phospholipases (PLAs) cleaving either *sn1* or *sn2* chain, and a recacylating enzyme [15, 213, 214]. The remodelling process may also include transfer of a fatty acid from one PL to another in a process referred to as transacylation [215]. For example, arachidonic acid can be first incorporated in PC by acyl-CoA-lysophoshatidylcholine acyltransferase (LPCAT) [215] and is then transferred from PC to lyso-PE by CoA-independent transacylase (CoA-IT). The PLA2 enzymes, especially the iPLA2 enzymes, play an important role by providing a stable pool of lysophospholipids as acyl chain acceptors [216-218].

3.3.1. Phospholipases

Two types of PLAs are involved in the remodelling of phospholipids, namely the PLA1s and PLA2s cleaving the *sn1* or *sn2* acyl chain, respectively.

The PLAs targeting the *sn1* linkage (PLA1) are poorly known. PLA1 activity has been detected in fungi, bonito muscle, bovine brain and testis, hornet venom and rat platelets [219]. The PLA1s mostly target PC molecular species, but the PLA1s found in rat platelets and bovine testis target PS and PA, respectively [220, 221].

Numerous different proteins with PLA2 activity have been recognized [222, 223] and can principally be divided into five groups; sPLA2s, cPLA2s, iPLA2s, PAF acetylhydrolases and lysosomal PLA2s [213, 223]. sPLA2s are secreted poteins and require Ca^{2+} for catalysis [223]. sPLAs have been found in various sources including snake and scorpion venoms, pancreatic juices, arthritic synovial fluid and several mammalian tissues [213]. The mammalian sPLA2s do not show preference regarding the acyl chains, but may have some head group specificity [213]. Typically sPLA2s possess high activity towards anionic phospholipids [224].

cPLA2s are large cytosolic proteins and the first member found of this group (GIVA PLA2) required calcium for translocation to a membrane [225-227]. The cPLA2s have been implicated in acyl chain turnover of phospholipids in cells stimulated via surface receptors. The cPLA2 members differ from each other in regard to their regulation by calcium [228-231]. GIVA PLA2 is the only PLA2 that exhibits a clear preference towards AA in the *sn2* position [232, 233]. It has also lysophospholipase as well as transacylase activities [232-234]. Its activity is regulated by calcium which makes the enzyme bind to a membrane via its C2 domain thereby stimulating its activity [235-239]. The GIVB and GIVC PLA2s have only modest specificity for the *sn2* acyl chain [230, 240]. GIVD, -E and F cleave AA and linoleic acid with equal efficiency and GIVF prefers PE over PC [228].

The enzymes of the iPLA2/GVI group do not require Ca^{2+} for their activities. The GVIA1 (iPLA2β) is in the cytosol, but upon stimulation translocates to the nucleus, ER, Golgi and mitochondria [241]. GVIB PLA2 (iPLA2γ) has been localized to the ER, mitochondria and the peroxisomes [242, 243]. The role of GVIA1 and GVIA2 in mammalian phospholipid homeostasis is dependent on the cell type [244-247]. GVIA was originally purified from macrophages and subsequently from several different sources [248-250]. This enzyme is in addition expressed as multiple splice variants, thereby increasing

the complexity of the GVIA PLA2 family [251]. Four other members of the GVI group have been recently found, i.e. neuropathy target esterase (GVIC), adiponutrin/PNPLA3 (GVID), TTS-2.2 (GVIE) and GS2 (GVIF) [252, 253].

The enzymes of the GVI group (mainly GVIA1 and GVIA2) produce free AA, lysophospholipids and lyso-PA and have transacylase and lysophospholipase activity [216, 223, 254]. They seem also to participate in phospholipid remodelling and PC homeostasis [216, 255]. However, studies in INS-1 cells, a model for pancreatic islet β- cells secreting insulin, have suggested that GVI PLA2 enzymes are more important in cell signalling than in remodelling of phospholipids [255, 256].

The PAF acetylhydrolases are divided into two groups, i.e. GVII and GVIII, and both of them hydrolyze the *sn2* acyl chain of PAF. PAF acetyl hydrolase GVIIA cleaves acyl chains up to 9 carbons long from the *sn2* position of oxidized lipids [213]. The PLA2 activity has been shown to decrease with increasing chain length of the *sn1* acyl chain [257]. The GVIIA is the only PLA2 that has been shown to be able to operate in the aqueous phase [257, 258].

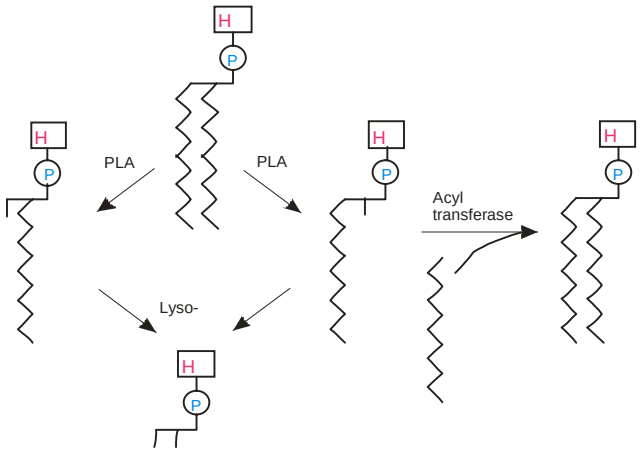

Figure 4. *Routes for phospholipid remodelling* – Phospholipid remodelling is a coordinated action of phospholipase and acyltransferase/transacylase activities. Simplified, either the *sn1* or *sn2* position acyl chain is cleaved off by a PLA1/PLA2, respectively. Thereafter a new acyl chain is attached by acyltransferase activity.

The fifth group consists of one enzyme, 1-O-acylceramide synthase (ACS), it has been purified from bovine brain. It esterifies the C-1 position of ceramide by acyl chains derived from phospholipids [259]. The ACS possesses both PLA2 and transacylase activities [260]. ACS enzyme has been shown to preferentially hydrolyze PC and PE species [259].

3.3.2. Acyl transferases

Acyl chain remodelling of phospholipids requires hydrolysis by a PLA and subsequent reacylation by an acyl transferase (Fig. 4). The exchange of acyl chains may also be mediated by a transacylase. While only few transacylases are known, several acyltransferases are involved in remodelling. The acyltransferases are divided into several groups according to their (primary) lysophospholipid acceptor, e.g. LCLAT (lyso-cardiolipin acyl transferase).

Acyltransferases possibly involved in phospholipid, PE and PS, remodelling are LPCAT3, LPCAT4, LPEAT1. All three possess LPEAT activity while the latter two also have LPSAT activity. In addition

the LPCAT3 and 4 display LPCAT activity [261, 262]. The LPCAT3 shows a preference for polyunsaturated acyl chains and 18:2-CoA whereas the LPCAT4 and LPEAT1 prefer the 18:1-CoA.

LPCAT1 catalyzes the synthesis of dipalmitoyl-PC and exhibits LPGAT activity [263, 264]. It is mostly expressed in the lung tissue [264]. In addition to LPCAT and LPGAT activity the enzyme has been shown to also catalyze PAF synthesis with a preference for 18:2- and 18:3-CoAs [263, 264]. LPCAT2 possesses LPCAT activity with a preference towards 20:4-CoA, but it is primarily involved in PAF biosynthesis and remodelling [265]. LPIAT1 has only been shown to catalyze the incorporation of AA and eicosapentaenoic acid to lysoPI [10].

4. Intracellular trafficking of aminophospholipids

4.1. Phosphatidylserine

Transport of PS (NBD-PS) from the PM to subcellular organelles is not vesicle-mediated since it was rapidly and extensively transported into subcellular organelles even when the temperature was lowered to 7 °C [40]. Translocation of labelled PS to mitochondria can be followed by monitoring formation of labelled PE since in mammalian cells PS decarboxylase is found only in the mitochondria and because that transport rather than decarboxylation proper is the rate-limiting step [81, 82]. Translocation of pyrene-labelled PS molecular species has been studied in BHK cells and was found to be dependent on the hydrophobicity of the PS molecular species, i.e. the more hydrophobic species are translocated more slowly, suggesting that the transport is due to spontaneous diffusion [266]. However, addition of a partially purified calcium-binding protein, S100B, to permeabilized CHO-K1 cells enhanced translocation of PS to mitochondria presumably due to an increased number of contact sites between MAM and the mitochondria [267].

Transport of PS from ER to mitochondria has been shown to be independent of cytosolic proteins in permeabilized CHO-K1 cells [81-83]. However, transport of PS to mitochondria was inhibited by depletion of ATP and cycloheximide poisoning [268]. ATP dependence of the transport process has been shown in experiments with permeabilized cells showing that the ATP required for transport is not connected to the ATP related to PS synthesis [81-83]. ATP may be required for the release of PS from the MAM fraction and therefore needed in the transport process [83, 208]. Depletion of cellular ATP caused accumulation of PS into the near vicinity of the mitochondria and also inhibition of PS decarboxylation [208]. However, in *in vitro* experiments with purified mitochondria and liposomal donors or isolated microsomes the transport and subsequent decarboxylation was independent of ATP [81, 82].

It has been estimated that in HeLa cells ~20% of the mitochondrial surface would be in contact with the ER [269]. Co-isolation of MAM with mitochondria from rodent livers has implicated that there is an association between these organelles [112, 270]. Indeed, morphological studies have revealed sites of close apposition or contact sites [269, 271]. One of the proteins suggested to maintain these MAM-mitochondria contact sites is Mitofusin 2 which has been shown to be required for calcium uptake into mitochondria [104]. Contact sites may also be involved in the transport of PS between the outer and inner mitochondrial membranes [210, 272, 273]. It has been proposed that transport of PS from MAM to mitochondria is assisted by close proximity or contacts between these organelles [112, 270]. Subcellular fractionation of CHO cells labelled with ^{3}H-serine revealed that the labelled PS was located both in the ER and MAM prior to decarboxylation and that it after depletion of ATP accumulated in MAM [208].

The role of the contact sites in PS transport is supported by that trypsin treatment of mitochondria, i.e. reduction of MAM-mitochondria contacts, as well as reduction of contacts between the outer and inner mitochondrial membranes by dinitrophenol, inhibit transfer and subsequent decarboxylation of PS [111, 274]. However, results from experiments with proteinase treatment of mitochondria with either trypsin or proteinase K have been inconsistent since in some studies either significant or no effect on uptake and decarboxylation of PS has been observed [3, 81]. Mutant CHO-K1 cells, which have normal PS synthesis and functional PSD, but defective decarboxylation have been found [275]. This deficiency appears to be due to a lesion in transport of PS from OMM to IMM, but the details of the defect remain unidentified [275].

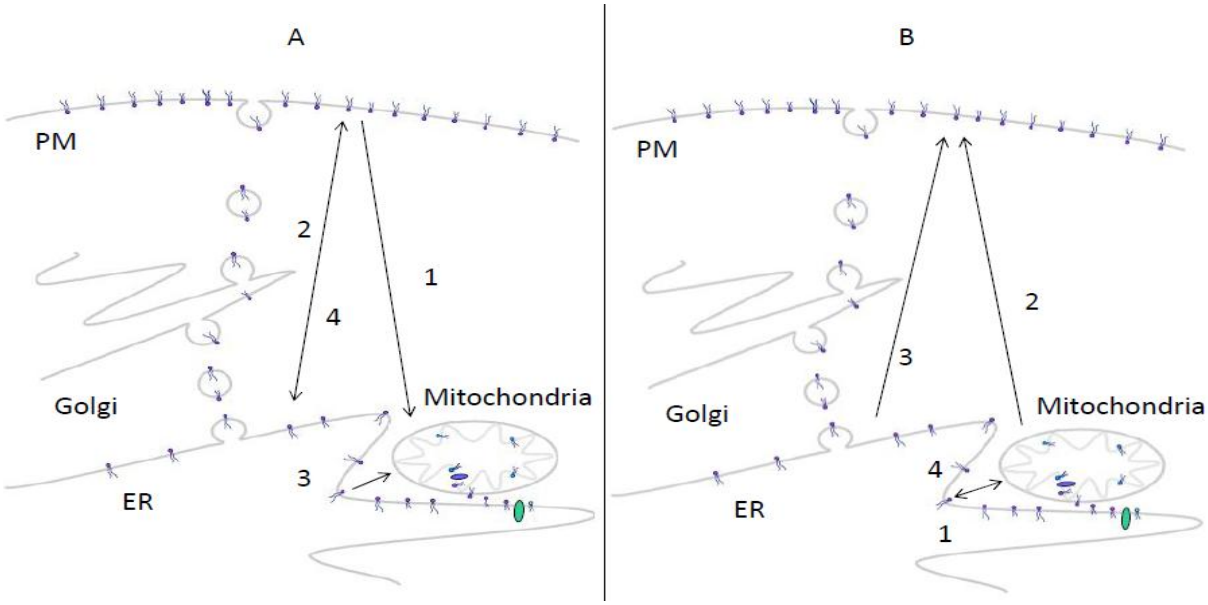

Figure 5. *Theoretical translocation routes for PS (A) and PE (B)* – PS may be transported from the PM directly to the mitochondria (1) or via ER (2) to mitochondria (3) for subsequent decarboxylation. Newly synthesized PS is translocated from the ER to mitochondria (3) or to other cell compartments such as the PM (4). PE is transported from mitochondria to the ER (1) and other cellular compartments (2) or alternatively from the ER to the PM (3) or in small amounts to mitochondria (4).

4.2. Phosphatidylethanolamine

PE is synthesized in mitochondria and ER, and is yet present in all membranes. Thus PE must be translocated from these organelles to others. PE, similarly to PC, has been shown to be transported from its site of synthesis (mainly the ER) to the PM, even in absence of ATP [78, 79]. Cells labelled with ^{3}H-ethanolamine showed that newly synthesized PE is transported to the PM without a detectable lag and that in pulse-chase experiments the transport continued for up to 2 h [79]. Transport of newly synthesized PE is kinetically very similar to that of PC, i.e. it is rapidly transported to the PM [79]. In contrast to the transport to the PM, the transport from microsomes to the mitochondria was found to be up to 80- fold slower for PE than for PC [276]. Transport experiments with isolated microsomes and mitochondria from rat liver tissue displayed a sequential transfer of PE from microsomes first to the outer and then to the inner mitochondrial membrane [277-279]. In CHO cells labelled with ^{3}H-ethanolamine, the resulting PE specific radioactivity was 10 times lower in mitochondria than in the microsomes after 2 h and practically no radioactivity was seen in the inner mitochondrial membranes [208]. Similarly, when mitochondria were exposed to trinitrobenzene or when the outer mitochondrial

membrane was removed with digitonin the imported PE was found primarily in the outer mitochondrial membrane [280].

PS derived PE may constitute up to 80% of the total cellular PE pool which indicates translocation from mitochondria to other cellular compartments [210]. The translocation of PE from the inner to the outer mitochondrial membrane has been shown to occur rapidly [274]. However, PE formed in mitochondria is suggested to be essential for the function of the organelle [208, 209].

In addition, there is clear evidence that the PE produced in mitochondria is transported to ER and PM [80, 208]. Transport of PE from mitochondria to PM in rat hepatocytes does not seem to be vesicle-mediated, since it is not inhibited by the disruption of the Golgi by Brefeldin A, but the detailed mechanism is not known [80]. Transport of the mitochondrial PE to the PM is very rapid and exceeds that of PE synthesized in the ER [80].

5. Tools to study metabolism and intracellular trafficking of phospholipids

5.1. Radiolabelled precursors

In order to study metabolism of newly synthesized phospholipids, labelled precursors have to be employed. Traditionally phospholipid synthesis and turnover has been investigated using radiolabelled precursors (e.g. ^{3}H-serine or ^{14}C-ethanolamine) [208, 268]. However, the use of radiolabelled precursors is problematic when studying individual phospholipids molecular species, since one has to separate the species by HPLC and determine the radioactivity and phosphate content of each fraction, which is laborious and insensitive.

5.2. Stable isotope-labelled phospholipids

The most recent advance in studies of phospholipids has been the synthesis of isotope-labelled phospholipids. The isotope labels, usually deuterium, may be located in the polar head group, in the acyl chains of the lipid in question or in both positions simultaneously. These phospholipids are chemically and physically nearly identical to endogenous phospholipids, since the only difference is the number of neutrons present in H or C. The isotope label is also an effective way to separate endogenous and labelled species by mass spectrometry, thus providing detailed data.

The combination of labelled precursors and mass spectrometric methods has been used for example to study the composition and dynamic remodelling of endonuclear glycerophospholipids in cultured cells [281]. By using isotope-labelled precursors pre-existing and newly synthesized PCs could be distinguished by alternative precursor ion scans [282]. In addition isotope-labelled methionine, that incorporates into PE by methylation, has been used to study the translocation of PC between ER and mitochondria in yeast [283], while isotope-labelled ethanolamine and choline have been used to study PE and PC metabolism in rat hepatocytes [284].

It is also possible to introduce the label in acyl chain moieties. This allows one to follow incorporation of a distinct acyl chain into subcellular organelles as well as to study phospholipid remodelling. The remodelling process has been studied for example in the synthesis of lung surfactant and PC synthesis in the liver tissue [285-287].

5.3. Fluorescent and spin-labelled lipid analogs

Fluorescent analogues of phospho- and sphingolipids have provided useful information on lipid translocation in both cytological and biochemical studies [68]. In addition the NBD-analogues of PC, PE, PS, PI, PA, SM, glucosylceramide and ceramide have been used to study lipid transport in cells [40, 75, 288-291]. Spin-labelled phospholipids have also been used for lipid transbilayer transport studies [29, 292]. The fluorescent analogs provide means to visually follow e.g. translocation of PLs. However, both the NBD- and BODIPY labelled analogues are more hydrophilic than the corresponding endogenous PLs thus resulting in data that does not fully correspond to the "natural" conditions [293, 294]. Pyrene-labelled phospholipid analogues resemble their natural counterparts more closely than NBD- and BODIPY-analogues [295], but also they carry an unnatural group that may cause aberrant behaviour.

5.4. Carriers of exogenous phospholipids

Labelling cells with phospholipids with very short acyl chains (i.e. low hydrophobicity) does not require additional carriers for effective transfer into cells. However, short-chain phospholipids spontaneously translocate between intracellular organelles and can be rapidly degraded. Lysophospholipids rapidly translocate to cells and are subsequently acylated therein, but the acylation produces multiple molecular species, thus complicating data interpretation.

Phospholipids which are equally hydrophobic as the endogenous ones do not spontaneously transfer to cells and thus carriers are needed. In principle the most delicate approach is to use lipid transfer proteins, but impractically high amounts of the protein are required for adequate labelling [296, 297]. Also other approaches, such as cells labelled with lipid vesicles containing a viral receptor have been used, but these turned out to be impractical due to complications [298].

Cyclodextrins are cyclic oligosaccharides with a hydrophobic cavity and can carry cholesterol and phospholipids [299-301]. Certain cyclodextrins transfer even efficiently hydrophobic phospholipids, thus making them potential carriers when introducing exogenous phospholipids to cells [266, 294, 302]. However, cyclodextrins can deplete cholesterol from cells and thus compromise their viability.

5.5. Monitoring of interorganelle translocation

Intracellular translocation of phospholipids may be studied by labelling cells with fluorescent phospholipid analogues and subsequently following the distribution of fluorescence in subcellular compartments. However, the fluorescent analogues do not fully behave as the corresponding endogenous PLs. Alternatively, one can exploit site-specific reactions, such as PS decarboxylation to study interorganelle translocation of PLs [303] or conduct subcellular fractionation [87, 208].

5.5.1. Subcellular fractionation

Intracellular transport of phospholipids may be studied by metabolic labelling of PLs combined with subcellular fractionation [87, 208]. However, impurity of the separated organelles is a major problem. For example endosomes (50% yield) with only a 5% contamination by ER membranes can contain up to 50% of ER derived lipids due to the 10-fold greater abundance of ER membranes [304]. The purity

of fractions may be increased by including additional steps, but this reduces the yield and increases the risk of losing subfractions of the organelle [14].

The crude mitochondrial fraction obtained by differential centrifugation is contaminated by lysosomes and the ER membranes (MAM). MAM may be removed by centrifugation in a Percoll density gradient [305, 306] or by protease treatment disrupting contact sites between mitochondria and MAM [111, 307, 308]. The mitochondria can be further subfractionated to the outer and inner mitochondrial membranes by digitonin treatment followed by centrifugation [274, 309].

5.5.2. Site-specific modifications

In mammalian cells formation of PE from PS can be used to follow the transport of PS to mitochondria, since the PS decarboxylase is located explicitly in the inner mitochondrial membrane and since transport is the rate-limiting step rather than decarboxylation proper [166, 202, 310].

Transport of PS derived PE from mitochondria to the ER may be followed by methylation of PE to corresponding PC by the PEMT located in the ER, with the bulk activity localized to the MAM [112, 311]. However, this approach can be used only in cells that express PEMT activity, such as hepatocytes or PEMT overexpressing cells [112, 311].

5.6. Elucidation of the phospholipases involved in PL metabolism

5.6.1. Pharmacological inhibitors

The function of individual PLA2 proteins involved in phospholipid metabolism has been studied extensively by using pharmacological inhibitors that act specifically on the different PLA2 proteins [312, 313].

Commonly used inhibitors of GIVA PLA2 (cPLA2α) are arachidonyl trifluoromethylketone (ATFK), pyrrophenone and methyl arachidonyl fluorophosphonate (MAFP). Neither ATFK nor MAFP are 100% specific thus complicating data interpretation [314-316]. The most potent inhibitor of GIVA PLA2 is pyrrophenone [317, 318].

Bromoenollactone (BEL) is the most commonly used inhibitor of iPLA2. The S- and R-enantiomers of BEL display partial specificity towards iPLA2β (GVIA) and iPLA2γ (GVIB), respectively [319]. However, BEL is not fully specific for iPLA2, but inhibits also e.g. PA phosphatase [320, 321].

5.6.2. RNA interference

The most attractive method available for inhibition of phospholipases is RNA interference. siRNA (or anti-sense oligonucleotides) have been used to knock-down GVIA PLA2, GVIB PLA2, GIVA PLA2, GIII PLA2, GX PLA2 and GVIII PLA2 [216, 322-326]. In addition, knock-out mice models may be generated as done for example for GVIA PLA2 [327, 328].

5.7. Mass spectrometry

Mass spectrometric lipidomic methods have developed rapidly in recent years. Combining stable-isotope labelling with mass spectrometry has added an important dimension to lipidomics [284, 329, 330].

There are two principal ways to conduct mass spectrometric analysis of lipids, i.e. tandem-MS or LC-MS (see below). For ion separation various analyzers may be used [331], ion-traps for full scan mode, i.e. scan for a range of masses (*m/z*), and quadrupole analyzers for multiple reaction monitoring [215, 329, 332]. The analyzers filter the ions based on their *m/z* (mass-to-charge-ratio) by adjusting the electrical field by DC (direct current) and RF (radio frequency) potentials of the analyzers [331].

The data obtained from mass spectrometry needs to be further analyzed. Internal standards should be included in samples for enabling quantitative analysis, and for the analysis itself specific software have been developed that take into account factors such as variations in ionization efficiency and deisotoping effects [333-336].

5.7.1. Direct infusion (DI) and class-specific scanning

Direct infusion combined with class-specific scanning (neutral loss or precursor-ion scanning) enables detection of different lipid classes and species with high sensitivity and specificity [337, 338]. The precursor ion scanning is achieved by direct infusion with subsequent scanning of characteristic ions acquired by fragmentation (ESI-MS/MS) [338-340]. The first quadrupole of a triple quadrupole scans for a range of *m/z* for glycerophospholipids, the second chamber then fragments the ions, selected in the first one, by collision-induced dissociation (CID). Finally the third quadrupole transmits a fragment with a specific *m/z* so that only ions producing the specific fragment are detected [341, 342]. For example, PC species can be detected by scanning for the precursor with a *m/z* of 184 (phosphocholine) [343, 344]. PS and PE can be detected by scanning for neutral loss of 87 or 141, respectively.

Compared to LC-MS, direct infusion has the advantage of constant electrospray conditions, but isobaric species may not be quantified without subsequent fragmentation. In addition, the sensitivity is not adequate for quantification of minor species [345].

5.7.2. Liquid chromatography- mass spectrometry (LC-MS)

Combination of normal phase liquid chromatography (LC) with masspectrometry offers an alternative way of analyzing phospholipids. In normal phase LC the phospholipids are separated by their head groups, i.e. the PL classes are eluated at different times, while the reverse phase LC separates molecular species by their acyl chain residues [334, 346]. Triple quadrupole analyzers, in the context of LC-MS, also allow sensitive monitoring called MRM (multiple reaction monitoring). LC-MS is more sensitive than direct infusion since the impurities that might cause suppression are separated. However, use of LC-MS increases the time needed for analysis as well as complicates the quantification due to changes in eluent composition which in turn affect the ionization efficiency [347].

DI-MS/MS and LC-MS allow the detection of both labelled and unlabelled lipids selectively [284, 337, 338]. Several stable-isotope labelled precursors may be used simultaneously using head group specific scanning. For example synthesis of D_4-PE and D_9-PC may be studied simultaneously by labelling with D_4-ethanolamine and D_9-choline and applying specific head group scans, neutral loss of 145 and precursor ion scan of 193, respectively [348].

AIMS OF THE CURRENT STUDY

The main goal of the current study was to elucidate factors contributing to aminophospholipid remodelling and intracellular translocation in mammalian cells. More specifically, we studied:

- Introduction of intact stable-isotope labelled phospholipids to cultured cells using cyclodextrin as a carrier.
- Pathways and kinetics of remodelling of exogenous phospholipid species in cultured cells and the A-type phospholipases involved in this process.
- Import of phosphatidylserine and -ethanolamine to mitochondria and export of phosphatidylserine-derived phosphatidylethanolamine from mitochondria.

EXPERIMENTAL PROCEDURES

Lipids and reagents **–** The unlabelled lipids and cholesterol were obtained from Avanti Polar Lipids (Alabaster, AL). The PE and PS species with a deuterium-labelled head group were synthesized from corresponding PC species and D-ethanolamine or D-serine by using phospholipase D mediated transphosphatidylation [349], except with a five-fold reduced reaction volume. The deuterium-labelled PC species were synthesized from corresponding PE species by methylation with D-methyl iodide [350]. The products were purified by normal phase HPLC [351], the purity was confirmed with mass spectrometry, and the concentration determined by phosphate analysis [352]. Sources of other chemicals are found in the original publications I-III.

Cell culture **-** Baby hamster kidney (BHK21) and HeLa cells were grown in cell culture flasks (Nunc, Roskilde Denmark) in DMEM (Gibco 41965) supplemented with 10% fetal bovine serum, 2mM glutamine, penicillin (200 units/ml) and streptomycin (200 ug/ml) under 5% CO_2 and 37°C. Wild type CHO-K1 cells were grown in HAM F-12 medium (Gibco 21765) with supplements as above. Human skin fibroblasts were grown in RPMI medium (Gibco) supplemented with 15% fetal bovine serum, 2 mM glutamine, penicillin (200 units/ml) and streptomycin (200 ug/ml). All cell culture media and supplements were from Gibco BRL (Life Technologies Inc. San Diego). For labelling, cells were plated 2-3 days prior to the experiment. The labelling and chase media were supplemented with 2% fetal bovine serum.

Labelling cells with stable-isotope labelled precursors **-** Confluent monolayers of BHK21 or HeLa cells were washed once with PBS and then labelled with stable-isotope labelled serine or ethanolamine as specified in the original publications. In some experiments hydroxylamine (1.5 mM), an irreversible inhibitor of the PS decarboxylase [167, 210], was added to the culture medium. After the pulse, the cells were washed with PBS and chased in DMEM in the presence or absence of unlabelled serine (3 mM) and/or ethanolamine (1 mM). Cells were then washed 4 times with PBS and scraped into 250 mM sucrose or H_2O.

Introduction of labelled phospholipids to cells **-** Donor vesicles composed of a labelled phospholipid species, palmitoyl-oleyl-phosphatidylcholine and cholesterol (1:4:5 or 1:9:10 mol/mol) were introduced to cells in DMEM. A small amount (0.1%) of 22:0/22:0 phosphatidylcholine species was included in the donor vesicles to assess their binding to cells. Methyl-β-cyclodextrin (2-10 mM) was used as carrier to enhance the transfer of phospholipids from donor vesicles to cells.

Lipid extraction **-** Lipids were extracted according to Folch et al. [353] except that 0.1 M HCl was used as the aqueous component and silylated glass tubes were used to maximize the recovery of PS. A mixture of internal standards was added at the single-phase stage of extraction for mass spectrometric quantification of the lipids. After evaporation of the solvents, the lipids were dissolved in chloroform/methanol (1:2, v/v) and stored at -20°C before the analysis**.**

Mass spectrometry and data analysis **-** After addition of aqueous NH_3 (4%), the sample was infused at 6 µl/min to the ESI source of a Micromass Quattro Micro triple-quadrupole mass spectrometer (Waters), operated as previously [334]. The D_9-PC, D_4-PE, D_3-PS and $D_3{}^{15}$N-PS species and unlabelled PC, PE, PS, SM and phosphatidylinositol species were selectively detected using head group-specific precursor or neutral loss scanning [284, 354]. The species were identified and

quantified using the LIMSA software [333]. For identification of the acyl chain *sn*-positions of the endogenous species, as well as the remodelling products derived from the exogenous species, PE and PS classes were isolated by normal phase HPLC [351], and the individual species were then subjected to collisionally activated dissociation and product ion analysis [338, 355, 356].

Cell integrity - In the Trypan Blue exclusion test, the cells were first detached from culture dishes by Trypsin-EDTA and then pelleted by centrifugation. Trypan Blue (0.4%) in PBS was added to the pellet and the stained (leaky) cells were counted using a hemocytometer. To assess cell viability, cells were incubated with Alamar Blue in DMEM at 37 °C for 2 h. Then 200 ul of the media was pipetted to a 96-well plate and the fluorescence intensity was measured on Cary Eclipse fluorometer with the excitation and emission wavelengths set to 530 and 590 nm, respectively. The control samples included *i*) cells incubated in DMEM, *ii*) cells killed with 70% ETOH, *iii*) cells killed with 5 mM $CuSO_4$ and *iv*) DMEM. Protein content was determined by a fluorometric method employing fluorescamine [357] or by the method of Lowry et al. [358]. DNA was quantitated by using the Quant-iT dsDNA Broad-Range Assay Kit (Molecular Probes) and cholesterol was determined with a cholesterol oxidase method [359].

Cell fractionation - HeLa cells grown on 14 cm dishes were detached from the dishes by Trypsin-EDTA and after washing with PBS and centrifugation (1500g, 2 min), the cells were suspended in 1ml of a mannitol (225 mM) - sucrose (75 mM) -Tris-HCl (30 mM) buffer, pH 7.4 and homogenized by passing 10 times through a 27G needle.

A crude mitochondrial fraction was obtained by differential centrifugation as described [208, 360] and was further fractionated into the inner mitochondrial membrane (IMM) and the outer mitochondrial OMM fractions [309], except that 2 mg of digitonin was used per mg of mitochondrial protein. The supernatant from the crude mitochondrial fraction was further centrifuged at 20800 x g in order to pellet all debris. This supernatant contained the microsomes and cytosol (microsomal fraction).

Immunoblotting – Western blotting with the following antibodies was used to assess the purity and cross-contamination of the subcellular fractions: calnexin (BD Biosciences) for ER/MAM; ACSL4 (Santa Cruz) for MAM; LAMP2 (Developmental Studies Hybridoma Bank) for lysosomes; VDAC (Calbiochem) for OMM and COXII (Santa Cruz) for IMM. Samples were pipetted on a 10% SDS-PAGE gel and transferred onto PVDF (Millipore) membranes. The target proteins were detected by using HRP-conjugated secondary antibodies and ECL+ (Amersham) detection reagent.

RNA interference – Cells (300 000) were plated on 3cm dishes and siRNA treated with siRNA against Mitofusin 2 (MFN2), and\or Mitostatin (TCHP), or PSS1/2 proteins. Non-targeting siRNA was used as control. siRNAs were transfected without FBS using 5 ul of transfection reagent to a final consentration of 2 nM (MFN2, PSS1, PSS2) or 5 nM (TCHP) siRNA.

Antibodies against Mitofusin 2 (Sigma Aldrich) and Mitostatin (Abgent) were used to detect the effect of siRNA on protein level, while RT-QPCR was used to determine the mRNA levels of MFN2, TCHP, PSS1 and PSS2.

RESULTS AND DISCUSSION

(I) Introduction of intact phospholipids to cultured cells using meβ-CD as a carrier

Meβ-CD mediated transfer of intact phospholipids

An efficient method for the introduction of labelled phospholipids to cultured cells would be highly useful when studying PL remodelling and interorganelle translocation. Since certain CDs have been shown to efficiently transfer phospholipids from donor vesicles to cells, it appears they could be practical to use [266, 294]. Therefore we determined optimal labelling parameters in order to obtain efficient incorporation of exogenous phospholipids with similar hydrophobicity as the endogenous ones using meβ-CD as a carrier.

To asses the effects of meβ-CD concentration we incubated BHK21 cells with donor vesicles containing either 14:1/14:1-, 16:1/16:1- or 18:1/18:1-PS. We found that the transfer of different PS species was enhanced with increasing cyclodextrin concentration (Fig. 6). However, the optimal CD concentration depended on the hydrophobicity of the phospholipid to be transferred. The less hydrophobic species, 14:1/14:1-PS, was efficiently transferred at 4-6 mM meβ-CD while the more hydrophobic species, 18:1/18:1-PS, would apparently require higher meβ-CD concentrations (>10 mM) for efficient transfer. The transfer efficiency increased with time and maximal incorporation was usually obtained after 2-3 h. The results show that the transfer of exogenous phospholipids to cells depends greatly on the hydrophobicity of their acyl chains. The less hydrophobic the PL the more efficiently it is transferred by meβ-CD.

We also studied the influence of the polar head group, which as expected, had a rather small effect on transfer efficiency (I, Fig. 2). Next the efficiency of incorporation of exogenous phospholipids to different cell lines was studied and it was found to vary somewhat (I, Fig. 3). For example, various PE species were equally transferred to CHO, HeLa and HF cells, but less efficiently to BHK cells, whereas PS species were transferred more efficiently to CHO and HF cells than to HeLa and BHK cells.

In previous studies phospholipid metabolism has been studied by incubating cells with radiolabelled or stable-isotope labelled precursors [284, 361]. However, these approaches do not allow studies of, for example, remodelling of single PL molecular species since numerous PL species become labelled already during the pulse. To overcome this problem intact phospholipids should be introduced to cells. Alternatively, PL species with short acyl chains, 6:0 or 8:0, or lysophospholipids could be introduced to cells, but even this is problematic, since rapid and potentially different metabolism of the short chain species from that of “natural” ones and uncontrolled reacylation of the lysophospholipids take place [362-365]. If one wants to introduce exogenous PLs with similar hydrophobicity to that of endogenous PLs a carrier is needed since they spontaneously move poorly into cells. Theoretically, phospholipid transfer proteins are ideal carriers, but they are inefficient resulting in the need for impractical amounts of such proteins [296, 297]. Another option could be fusion of vesicles with virus receptors [298]. Of the different cyclodextrins, carboxyethyl-γ-cyclodextrin (γ-CD) has been previously used in the introduction of pyrene labelled PLs to cells [266]. Use of meβ-CD and donor vesicles enables efficient transfer of long-chain (phospho) lipids from vesicles to cells and, combined

with mass spectrometric analysis, lays a basis for detailed studies of phospholipid metabolism and trafficking.

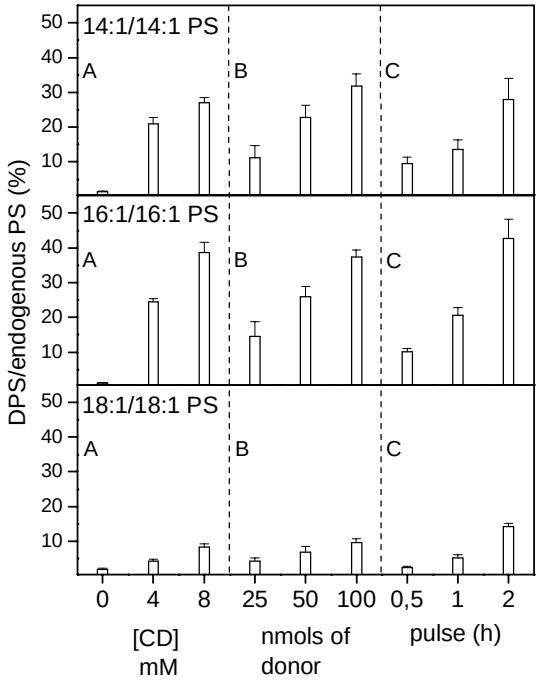

Figure 6. *Optimization of the labelling parameters* – The labelling conditions for exogenous D-PS molecular species with varying molecular hydrophobicity were examined in BHK cells. The figure displays how increase in CD concentration (lane A), donor amount (lane B) and pulse time (lane C) affect the transfer of exogenous PS species from donor vesicles into cells as well as illustrates the effect of hydrophobicity in transfer efficiency.

Risks in using meβ-CD as a carrier

Incubation of cells with meβ-CD and donor vesicles could compromise cell viability e.g. by depleting cholesterol. Therefore we studied how incubation with meβ-CD and donor vesicles affects cell integrity.

We found that cell intactness or viability was not compromised even when rather high meβ-CD concentrations (8 mM) were used. The morphology of the cells changed somewhat during the incubation and they were also more prone to detach from the dish than untreated cells. The growth of the "labelled" cells was similar to that of the controls based on protein and DNA determinations (I, Figs. 6 and 7).

Since meβ-CD is known to extract cholesterol from cells we studied if this can be prevented by addition of cholesterol in the donor vesicles. We incubated BHK21 cells with vesicles containing 0-50% cholesterol. We found that cholesterol depletion could be almost fully compensated by addition of 50% cholesterol in the donor vesicles (I, Fig. 5). Besides cholesterol depletion, meβ-CD may also deplete other lipids from cells. We observed some depletion of cellular sphingomyelin (SM), but the level of this lipid was normalized during the 24 h chase period (Fig. 7). The PC content of the cells was increased during the incubation with vesicles (which mainly consist of PC), but it was also normalized during a 24 h chase.

In previous studies meβ-CD has been described to deplete cholesterol as well as other lipids from cells [301, 366, 367]. Our observations are consistent with these studies. In addition meβ-CD has been reported to cause morphological changes in cells as well as cause leakage of LDH and inhibition of cell growth and cell cycle arrest [301, 368-370]. These harmful effects of meβ-CD on cells, beside morphological changes, were not seen at incubation times and meβ-CD concentrations needed for efficient transfer of hydrophobic exogenous PLs to cells, thus implying that this method does not affect the liability of obtained data.

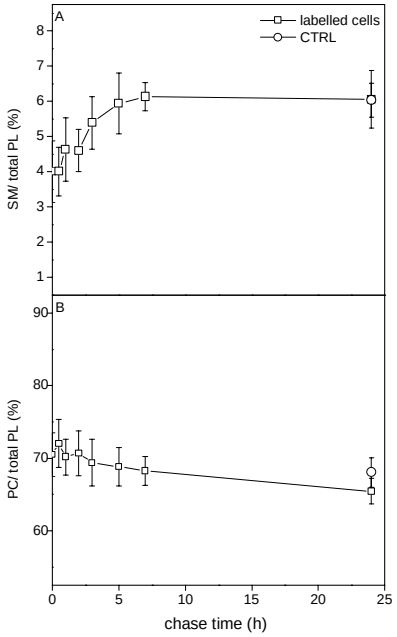

Figure 7. *Effect of meβ-CD mediated transfer of exogenous PLs to cellular SM and PC* – BHK21 were labelled with exogenous PL and 8 mM meβ-CD for 1 h and chased for 24 h. Panel A displays the relative amount of cellular sphingomyelin during the chase while panel B shows the relative amount of cellular PC expressed as % of total cellular PL.

(II) Remodelling of phosphatidylserine and phosphatidylethanolamine species

Remodelling of exogenous PE and PS

In theory all phospholipid molecular species could be synthesized *de novo*. However, many of the endogenous molecular species are made by remodelling of existing ones, i.e. by changing one or both of the acyl chains of the remodelled phospholipid. We studied the remodelling of different exogenous PS and PE molecular species in BHK21 cells using the meβ-CD mediated transfer and ESI-MS/MS analysis.

We found that remodelling of exogenous PE and PS species in BHK21 cells was dependent on both the acyl chains and the head group. PE and PS molecular species that are normally absent or only minor components of the cellular PE and PS pools were rapidly and extensively remodelled (Fig. 8). For example, both 14:1/14:1-PE and PS were rapidly remodelled to natural species such as 18:1/18:1-PE and 18:0/18:1-PS, respectively. In contrast, exogenous PE and PS species similar or identical to the major endogenous species, e.g.18:1/18:1 PE, were hardly remodelled at all.

We also found that the kinetics and sequence of *sn1* versus *sn2* remodelling were dependent on the acyl chains and head group. The PE species with a saturated acyl chain in the *sn2* position were very rapidly remodelled at this position, whereas similar PS species were remodelled only at a moderate rate. The effect of acyl chain *sn*-position was strikingly demonstrated by studies with positional isomers. For example, while 14:0/18:1-PE was remodelled slowly, its positional isomer, 18:1/14:0 PE, was very rapidly and extensively remodelled in the *sn2* position yielding several different species (II, Fig. 3). The *sn1* position of PE species was found to be preferentially acylated with 16:1 and 18:1 whereas in PS the preferred acyl chain was 18:0. Together these results indicate that the reacylation of PE and PS molecular species is conducted by different transacylases.

Previously it has been shown that phospholipids are remodelled at both *sn* positions [287, 371]. Our data is consistent with these findings as seen from remodelling of e.g. 14:1/14:1-PS which was extensively remodelled yielding species (e.g. 18:0/18:1) that are a result of acyl chain change at both *sn*-positions. In addition it has been shown that the *sn1* position of PS is preferentially acylated with 18:0 [372] as was also found in our results. This preference also explains why PS with 18:0, or PE species with 16:1 or 18:1 in the *sn1* position, were poor targets for PLA1 activity. In previous studies it has been shown that transacylase activity depends on the phospholipid head group [373, 374]. In agreement, the remodelling of corresponding PS and PE molecular species was found to be different. It is also known that endogenous PE normally do not contain saturated acyl chains at *sn2* position [24, 197] thus explaining the drastic difference in remodelling of PE positional isomers.

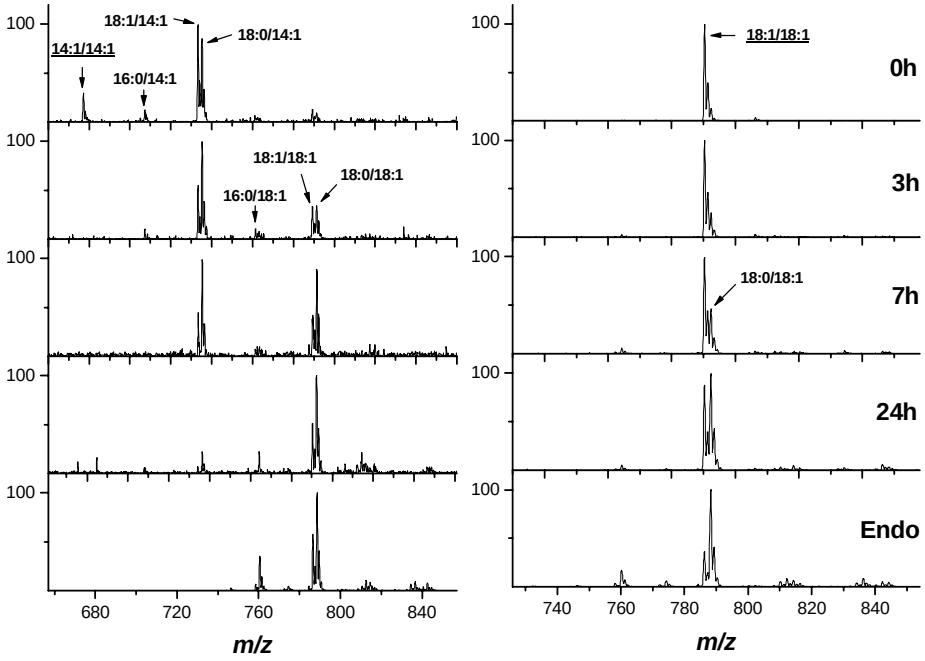

Figure 8. *Remodelling of exogenous PS molecular species* – BHK cells were labelled with 14:1/14:1 (left panel) or 18:1/18:1 D-PS (right panel) molecular species for 1 h and chased for the indicated times. In comparison spectra of the endogenous PS composition is shown in the bottom row.

Remodelling of exogenous PLs illustrates the remodelling pathways for different molecular species. However, direct comparison with the remodelling of endogenous PLs should be done cautiously since for example the kinetics may vary due to that mixing of the exogenous PL to the endogenous PL pool takes some time despite relatively rapid intracellular trafficking [375]. Study of PL remodelling is

made feasible by using meβ-CD mediated transfer of exogenous heavy-isotope labelled PLs to cells and mass spectrometric analysis. These tools provide detailed data on the remodelling pathways and kinetics of single molecular species.

PLAs involved in remodelling of PS and PE

The remodelling process involves PLA1 and PLA2 enzymes that hydrolyse the acyl chain from the respective *sn*-positions. The very different remodelling kinetics of corresponding PE and PS molecular species suggests that several different PLAs are involved in the remodelling of PE and PS. To evaluate the role of different PLA2s in PE and PS remodelling, inhibitors against iPLA2β (S-Bel, MAFP), iPLA2γ (R-Bel) and cPLA2 (MAFP) were employed.

We found that MAFP was the most potent inhibitor of PE and PS remodelling, while R-Bel and S-Bel were less effective (Fig 9.). S-Bel inhibited the remodelling of 14:1/14:1-PS more efficiently than of 14:1/14:1-PE, thus indicating different PLAs in remodelling of PE vs. PS. Studies with other molecular species supported the idea that that several PLAs, able to distinguish between acyl chains and head groups, participate in the remodelling process (II, Fig. 5). For example remodelling of 16:0/20:4-PS was not inhibited by any of the inhibitors while remodelling of 14:0/16:0-PS was almost completely inhibited by both R-Bel and S-Bel but not MAFP (II, Fig. 6).

We also studied the effect of Triacsin C, an inhibitor of acyl-CoA synthases (II, Fig. 6). Triacsin C did not affect PE remodelling, but intriguingly, inhibited remodelling of all PS species tested, thus suggesting that acyltransferases are involved in remodelling of PS but not PE. Lack of inhibition of PE remodelling by Triacsin C suggests that both *sn*-positions of this PL are reacylated by transacylases.

The data obtained by using inhibitors of cPLA2, iPLA2β and iPLA2γ support the role of these enzymes in the remodelling process of several PE and PS molecular species. However, the data suggests that also other, unidentified, PLAs participate in the remodelling process.

To date only little is known about enzymes with PLA1 activity, but several PLA2 enzymes have been implicated in the remodelling process [216, 217, 324, 376, 377]. Previous studies have shown that distinct PLA2s show preference for PE or PC [228, 378], thus supporting the data obtained in this study that PLAs display head group specificity. Although PLA2 inhibitors, such as Bel and MAFP, are useful when studying the role of phopholipases in PL remodelling the results should be evaluated with caution since these inhibitors are not completely specific towards iPLA2 and cPLA2, respectively [315, 320, 321]. An alternative would be the use of RNA interference in order to identify individual phospholipases responsible for PL remodelling [216, 325, 326].

Previous studies have shown that reacylation of the *sn2* position of lysoPS *in vitro* is conducted by acyl-CoA-dependent acyltransferases/transacylases [379, 380]. Our data is in agreement with these observations since Triacsin C inhibited remodelling of *sn2* position of PS (II, Fig. 6). However, the large variety of acyltransferases and their broad lyso-PL specificity complicates the task of specifying the single enzymes responsible for PL remodelling [261, 262].

The enzymes implicated in PL remodelling have been localized to several intracellular compartments including the ER, the Golgi, mitochondria, nucleus and peroxisomes [324, 376, 377, 381-387]. This intracellular distribution of the enzymes may contribute to the specific lipid compositions of organelles. The observed differences in remodelling of different PE and PS molecular species might

also be partially due to their differential intracellular trafficking and distribution [388]. In mammalian cells organelle specific remodelling has been found for PC [282]. Further studies are needed for elucidation of the speficic PLAs responsible for PL remodelling and for localization of the site of PL remodelling.

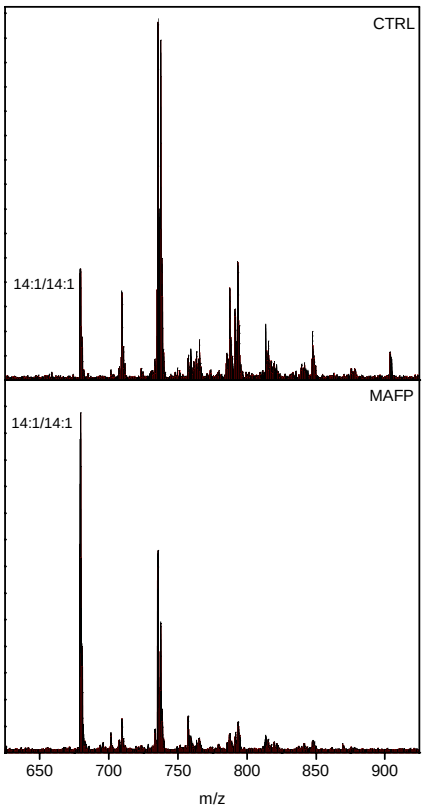

Figure 9. *The role of PLA2 enzymes in remodeling of exogenous PS* – BHK cells were labelled with 14:1/14:1 D-PS either with or without MAFP, an inhibitor of cPLA2 and iPLA2. The figure displays the situation after a 1 h pulse and a 30 min chase. The peak representing the introduced D-PS is indicated with a mark next to the peak. MAFP (lower panel) significantly inhibits remodelling of this PS molecular species as seen from the profiles of the spectra, i.e. relatively more of the precursor is present when MAFP was added and also the relative amounts of remodelling products (additional peaks) are smaller than without PLA2 inhibition.

(III) Trafficking of phosphatidylserine and phosphatidylethanolamine molecular species to and from mitochondria

Contribution of the PSD and Kennedy pathways to the cellular PE pool

The cellular PE is derived from two pathways, the PS decarboxylation pathway and the Kennedy pathway. The PSD pathway produces PE by decarboxylation of PS in mitochondria whereas the Kennedy pathway synthesizes PE *de novo*. To study the contribution of these pathways to the total cellular PE pool we incubated HeLa cells with heavy-isotope labelled serine or ethanolamine under various conditions.

When HeLa cells were incubated for 24 h with D_4-ethanolamine ~60%, of the total cellular PE pool consisted of the labelled species (Fig. 10). In contrast, 24 h incubation with labelled serine resulted in labelling ~20% of the total cellular PE pool. While these data seem to indicate that most of cellular PE is synthesized via the Kennedy pathway, analysis of the labelling of the intracellular serine and ethanolamine pools showed that the specific labelling of ethanolamine was significantly higher (up to 6-fold, depending on incubation time) than that of serine (III, Fig. 1). When this was taken into account, the difference in specific labelling of the cellular PE pool via the two pathways was significantly reduced indicating that ~66% of cellular PE was derived from the Kennedy pathway and the rest from the PSD pathway (III, Fig. 1).

Figure 10. *Specific labelling of the cellular PE pool by PSD and Kennedy pathways* – HeLa cells were incubated for 24 h with heavy-isotope labelled serine or ethanolamine. The figure shows the specific labelling of the total cellular PE pool via PSD and Kennedy pathways, respectively. Differences in specific labelling of cellular serine or ethanolamine pools are not taken into account.

We also found that the profile of the PE molecular species derived from PS differs significantly from the profile of PE species produced via the Kennedy pathway (III, Fig. 2). The most notable difference is that many polyunsaturated species (e.g. 38:4, 38:5 and 40:6) and the 36:1 species are more abundant in PE derived from the PSD pathway. On the other hand, the 32:1 and 32:2 species are more abundant in PE produced via the Kennedy pathway. The profile of the endogenous PE species is more similar to that of serine labelled than ethanolamine labelled PE species.

In earlier studies it has been shown that the contribution of the PSD and Kennedy pathways to the total cellular PE pool varies between cell lines as well as between intracellular organelles [3, 197]. In agreement, our data indicate that in HeLa cells the contribution by the Kennedy pathway to the PE pool is somewhat higher even when the higher specific labelling of the cellular ethanolamine pool is taken into account [82, 197].

It has also been shown that the PSD and Kennedy pathways provide cells with different PE molecular species [197]. Accordingly, in HeLa cells these two pathways seem to produce the same subset of PE molecular species, but in different proportions (III, Fig. 2). Interestingly, the cellular PE pool reminds us more of the PE composition derived from PSD pathway even though a majority of the cellular PE

seems to be derived from the Kennedy pathway. Why two different biosynthetic routes are needed is not clear but the PE from PSD pathway has been indicated to be crucial for the function of mitochondria [208, 209], thus explaining one function of the PSD route. In addition, it is known that mammalian cells cannot synthesize ethanolamine *de novo* and thus require supplementation of ethanolamine from culture media, from degradation of PE derived from PS, or to some degree from degradation of sphingolipids [187]. The restricted supply of ethanolamine could direct the synthesis of PE to be conducted via the PSD pathway, thus also potentially explaining the resemblance of the endogenous PE pool to that derived from the PSD pathway (III, Fig. 2). In accordance, we found that supplementating HeLa cells with ethanolamine stimulated synthesis of PE by the Kennedy pathway.

Import of PS to mitochondria and export of PE therefrom

A large part of the cellular PE pool is derived from the PSD pathway. In addition, in mammalian cells PS decarboxylase is explicitly located in the inner mitochondrial membrane. Thus, PS must be imported to mitochondria in order to be decarboxylated. To study this import process we first introduced D_4- labelled exogenous PS species to HeLa cells using meβ-CD as a carrier.

We found that the exogenous PS species were decarboxylated at very different rates. The 16:1/16:1 species was decarboxylated rapidly, i.e. close to 60% was decarboxylated during the first 2 h (Fig. 11 A). Although this species is less hydrophobic than the major endogenous PS species, e.g. 16:0/18:1 and 18:0/18:1, its efficient decarboxylation demonstrates that PSD has a high capacity to decarboxylate PS molecules entering the IMM. As expected the decarboxylation was less efficient for 16:0/18:1 apparent due to it being more hydrophobic than 16:1/16:1-PS. Surprisingly, the polyunsaturated 18:0/20:4 species was decarboxylated faster than predicted based on its hydrophobicity, i.e. its hydrophobicity should be close to that of 16:0/18:1.

We next studied the translocation of endogenous PS species. The cells were incubated with D-labelled serine for 4 h and then chased for a further 8 h. Major differences in the rate of decarboxylation among different PS species were found. The most rapid decarboxylation was observed for the polyunsaturated species 38:4 and 38:5, while the 34:2, 36:1 and 40:6 species were decarboxylated at a significantly slower rate (III, Fig. 3). No clear correlation between the rate of decarboxylation and the molecular hydrophobicity was found, but rather a dependence on the acyl chain composition.

Transport of PS to mitochondria has been indicated to occur via membrane contact sites between the MAM and the mitochondria. To study this we used RNA interference to knock-down Mitofusin 2 and/or Mitostatin proteins that have been indicated to participate in MAM-mitochondria contact sites in mammalian cells [104, 117]. However, we did not find any difference in decarboxylation between control and knock-down cells.

To study the export of PS derived PE from mitochondria we first labelled HeLa cells with exogenous D_4-PS species and then isolated the IMM and microsomal fractions. Translocation of PE species derived from exogenous PS from mitochondria to microsomes also varied significantly (Fig. 11 B). As can be seen in Fig. 11 B the most efficient translocation of the three molecular species presented, was observed for the di-16:1 species, i.e. ~20% of this species was found in microsomes at or soon after the end of the pulse. Interestingly the least efficient transfer was observed for the 18:0/20:4 PE species, which was imported as PS into mitochondria more efficiently than expected.

The export of PE derived from endogenous PS was studied by labelling HeLa cells for 3 h with D-labelled serine and subsequent isolation of IMM and microsomal fractions. After the 3 h incubation with D-labeled serine ~4% of the PE in IMM was labelled, while only ~1.5% of the microsomal PE pool consisted of the labelled species (III, Fig. 4). After 24 h of chase the amount of specific labelling in the microsomal fraction had increased to ~4%. The time-dependent redistribution of PS-derived PE from mitochondria to microsomes is clearly illustrated by the fact that during the chase the fraction of cellular D-PE in microsomes increased 2-fold (from ~9% to ~22%) while that in IMM decreased correspondingly from ~29% to ~13% (III, Fig. 4). These data demonstrate that PE derived from PS in mitochondria is effectively exported from mitochondria.

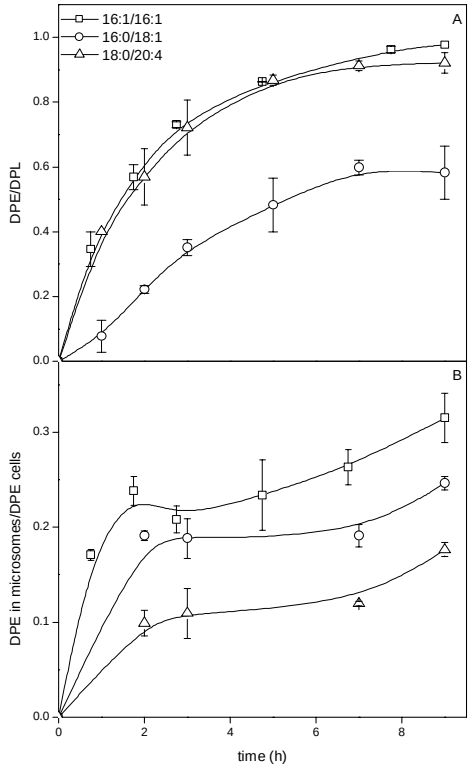

Figure 11. *Import of exogenous PS to mitochondria and export of PE therefrom* – HeLa cells were labelled with the indicated exogenous D-PS molecular species for 45 min (16:1/16:1) or 1 h and chased for up to 8 h and IMM and microsomal fractions were isolated. Panel A shows the relative amount of D-PE out of the total D-PL thus displaying the decarboxylation and translocation of the particular D-PS molecular species to mitochondria. Panel B displays the relative amount of indicated D-PE, derived from corresponding exogenous D-PS, in microsomes out of the total cellular amount of the particular D-PE molecular species.

All PS-derived PE molecular species were transported from IMM to microsomes, but at significantly different rates. The 32:2 (16:1/16:1) species was most efficiently transported, i.e. almost half of the cellular pool of this lipid was found in microsomes after a 24 h chase. Also the 34:2 and 38:6 species were translocated more efficiently than the remaining ones. Interestingly, in agreement with results obtained with exogenous PS species, the 18:0/20:4 molecular species was translocated only modestly even though it was efficiently decarboxylated (III, Fig. 5).

Previously it has been stated that, depending on cell type the majority of the cellular PE would be derived from the PSD pathway [80, 168, 194, 195, 208], thus indicating that PS is transported from its site of synthesis in ER/MAM [112, 166, 272] to mitochondria. This transport has been studied for example by using exogenous pyrene labelled PS species and ^{3}H-serine [81, 82, 87, 111, 167, 208, 266, 268, 270, 354]. It has been indicated that the transport is ATP-dependent, that newly made PS is preferentially translocated and that the transport rate of PS molecular species depends on the hydrophobicity [167, 266, 270, 330, 354]. Accordingly, we found that the less hydrophobic exogenous PS molecular species were decarboxylated, i.e. translocated to mitochondria, more efficiently than those with higher hydrophobicity, with the exception of the decarboxylation rate higher than expected for 18:0/20:4. However, this was not found to be the case with decarboxylation of newly synthesized PS species. We found that molecular species 38:4 and 38:5 were decarboxylated with higher efficiency than expected based on their estimated hydrophobicity (III, Fig. 3). When comparing mono- and diunsaturated molecular species with the same acyl chain length it was found that in general the species with two double bonds, i.e. less hydrophobic ones, were more efficiently decarboxylated. In earlier studies it has been suggested that the PSD pathway would preferentially produce distinct PE species and that the PSD would have some substrate specificity [197-200]. However, our data suggests that the PSD and Kennedy pathways produce the same PE species but in different ratios. The decarboxylation of newly synthesized PS species did not directly correlate with molecular hydrophobicity, but rather it was dependent on the acyl chain composition of the individual molecular species. The mechanism behind this remains unclear.

As stated previously, the translocation of PS to mitochondria is ATP-dependent, not vesicle mediated, but rather due to spontaneous diffusion [82, 167, 266, 268, 330, 354]. Since PS is synthesized in the ER, more closely in the MAM, it has been thought that PS could be translocated to mitochondria via close appositions between the MAM and mitochondria [87, 111, 112, 167, 208]. These contact sites are suggested to be maintained by proteins or protein complexes. Two proteins implicated to play important role in the contact site formation between the mitochondria and the MAM are Mitofusin 2 and Mitostatin [104, 117]. In fact, yeast cells with mutated proteins in the ERMES complex, a complex maintaining ER-mitochondria junctions, displayed a slower flow of phospholipids to mitochondria and lower abundance of mitochondrial PE [116]. However, the transport of PLs to mitochondria was not totally inhibited. Homologues for ERMES proteins in mammalian cells have not been identified thus complicating studies of the role of similar ER-mitochondria contact site complexes. However, the Mitofusin 2 and Mitostatin proteins implicated to participate in contact site formation in mammalian cells could be knocked-down by RNA interference. As seen from our results, Mitofusin 2 or Mitostatin do not appear to participate in ER-mitochondria contact site function mediating PS translocation to mitochondria (III, Supplement Fig. 3). To elucidate the role of contact sites in PS translocation to mitochondria, more contact site proteins need to be identified.

Only a few studies have addressed the export of PE from mitochondria although the majority of cellular PE could be derived from PS [208, 270, 389]. PE formed in mitochondria is translocated to ER and the transport is independent of cytosolic proteins and not stimulated by exogenous ATP [208, 270]. In agreement with these results our data confirm translocation of PE derived from PS to microsomes (III, Fig. 4). In addition our data indicated that there is selectivity in transport of different molecular species (III, Figs 5 and 6). However, the mechanism behind this selectivity remains to be elucidated.

Import of PE to mitochondria

Since mitochondria can synthesize PE via decarboxylation of PS they may in theory obtain their PE pool from this pathway only. Therefore we studied if PE synthesized by the Kennedy pathway is imported to mitochondria and what the contribution of this route is to the mitochondrial PE pool.

To study this we incubated HeLa cells with D_4-ethanolamine and isolated the IMM and microsomal fractions. We found that after a 1 h pulse, ~60% of the cellular D_4-PE was found in microsomes and only ~2% in the IMM (III, Fig. 8). When the cells were then chased for 24 h in the presence of unlabelled ethanolamine, the microsomal fraction of labelled PE remained constant, while that in IMM doubled, but was still very low, i.e. ~4%. These data indicate that PE synthesized in the ER translocates poorly to mitochondria.

When the D_4-PE molecular species compositions of microsomes and IMM were studied they were found to be nearly identical. In addition, the time-dependent changes in D_4-PE compositions in the two fractions were close to being identical (III, Supplement Fig. 4). These data and the fact that the IMM fractions could not be totally purified from the MAM and lysosomal contaminants indicate that the actual amount of translocated D_4-PE is even smaller than calculated.

Previous studies have shown that PE synthesized by the Kennedy pathway in the ER [166, 190, 192] is transported to the PM in a similar fashion as PC and PS [79]. However, contradictory data have been presented about translocation of PE from ER to mitochondria [197, 208, 276]. Our data supports the suggestion that PE synthesized in the ER is transported to mitochondria inefficiently. In fact, as stated previously, most of the mitochondrial PE seems to be derived from the PSD pathway which also seems to be crucial for the function of the organelle [197, 208, 209].

CONCLUSIONS

Metabolism and translocation of PE and PS were studied. The following main conclusions were drawn:

- We showed that meβ-CD is an efficient carrier of exogenous phospholipids to cultured cells and that the developed method does not affect cell viability. The efficacy of meβ-CD mediated transfer of heavy-isotope labelled phospholipids is dependent on the molecular hydrophobicity, pulse time and cyclodextrin concentration. The vesicular cholesterol concentration is crucial in order to compensate cholesterol depletion by meβ-CD. The method developed enables e.g. extensive manipulation of cellular PL composition, and is thus useful when studying mechanisms contributing to PL homeostasis.

- By using heavy-isotope labelled phospholipids we showed that the remodelling pathways of PS and PE and their kinetics are highly dependent on the acyl chain composition as well as the polar head group of the phospholipid. The remodelling process is conducted by several different phospholipases targeting both *sn1* and *sn2* positions, in addition to acyltransferases and transacylases. The phospholipases are selective over different molecular species and phospholipid classes. Tha data obtained lays a basis for future studies to elucidate both the processes and the proteins involved in maintenance of PL homeostasis.

- The Kennedy and PSD pathways of PE synthesis provide the same molecular species but in different ratios. The import of PS to and export of PE from mitochondria are not directly related to the molecular hydrophobicity, but rather to the acyl chain composition of the PS and PE species. The import of newly synthesized PS to mitochondria is not affected by knock-down of the MAM-mitochondria contact site proteins Mitofusin 2 and Mitostatin.

- Heavy-isotope labelled precursors/phospholipids and mass spectrometry employing class-specific scans is a powerful tool to study phospholipid metabolism. Highly detailed data of time-dependent synthesis, remodelling and transport of single PL molecular species can be obtained by using this approach.

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REFERENCES

1. Ishii, S. and T. Shimizu, *Platelet-activating factor (PAF) receptor and genetically engineered PAF receptor mutant mice.* Prog Lipid Res, 2000. **39**(1): p. 41-82.
2. Shimizu, T., T. Ohto, and Y. Kita, *Cytosolic phospholipase A2: biochemical properties and physiological roles.* IUBMB Life, 2006. **58**(5-6): p. 328-33.
3. Vance, J.E. and R. Steenbergen, *Metabolism and functions of phosphatidylserine.* Prog Lipid Res, 2005. **44**(4): p. 207-34.
4. Kusumi, A., I. Koyama-Honda, and K. Suzuki, *Molecular dynamics and interactions for creation of stimulation-induced stabilized rafts from small unstable steady-state rafts.* Traffic, 2004. **5**(4): p. 213-30.
5. Sharma, P., et al., *Nanoscale organization of multiple GPI-anchored proteins in living cell membranes.* Cell, 2004. **116**(4): p. 577-89.
6. Marsh, D., *Lateral pressure profile, spontaneous curvature frustration, and the incorporation and conformation of proteins in membranes.* Biophys J, 2007. **93**(11): p. 3884-99.
7. Vance, J.E., *Phosphatidylserine and phosphatidylethanolamine in mammalian cells: two metabolically related aminophospholipids.* J Lipid Res, 2008. **49**(7): p. 1377-87.
8. Schlame, M., D. Rua, and M.L. Greenberg, *The biosynthesis and functional role of cardiolipin.* Prog Lipid Res, 2000. **39**(3): p. 257-88.
9. van Meer, G., D.R. Voelker, and G.W. Feigenson, *Membrane lipids: where they are and how they behave.* Nat Rev Mol Cell Biol, 2008. **9**(2): p. 112-24.
10. Yamashita, A., T. Sugiura, and K. Waku, *Acyltransferases and transacylases involved in fatty acid remodeling of phospholipids and metabolism of bioactive lipids in mammalian cells.* J Biochem, 1997. **122**(1): p. 1-16.
11. Ford, D.A., et al., *Lipidomic analysis of the retina in a rat model of Smith-Lemli-Opitz syndrome: alterations in docosahexaenoic acid content of phospholipid molecular species.* J Neurochem, 2008. **105**(3): p. 1032-47.
12. Hicks, A.M., et al., *Unique molecular signatures of glycerophospholipid species in different rat tissues analyzed by tandem mass spectrometry.* Biochim Biophys Acta, 2006. **1761**(9): p. 1022-9.
13. Garcia, M.C., et al., *Effect of docosahexaenoic acid on the synthesis of phosphatidylserine in rat brain in microsomes and C6 glioma cells.* J Neurochem, 1998. **70**(1): p. 24-30.
14. van Meer, G., *Cellular lipidomics.* Embo J, 2005. **24**(18): p. 3159-65.
15. Lands, W.E., *Stories about acyl chains.* Biochim Biophys Acta, 2000. **1483**(1): p. 1-14.
16. Hermansson, M., K. Hokynar, and P. Somerharju, *Mechanisms of glycerophospholipid homeostasis in mammalian cells.* Prog Lipid Res. **50**(3): p. 240-257.
17. Pomorski, T., et al., *Lipid distribution and transport across cellular membranes.* Semin Cell Dev Biol, 2001. **12**(2): p. 139-48.
18. van Meer, G., *Lipid traffic in animal cells.* Annu Rev Cell Biol, 1989. **5**: p. 247-75.
19. Kobayashi, T., et al., *Separation and characterization of late endosomal membrane domains.* J Biol Chem, 2002. **277**(35): p. 32157-64.
20. Matsuo, H., et al., *Role of LBPA and Alix in multivesicular liposome formation and endosome organization.* Science, 2004. **303**(5657): p. 531-4.
21. Strauss, J.F., 3rd, et al., *START domain proteins and the intracellular trafficking of cholesterol in steroidogenic cells.* Mol Cell Endocrinol, 2003. **202**(1-2): p. 59-65.
22. Colbeau, A., J. Nachbaur, and P.M. Vignais, *Enzymic characterization and lipid composition of rat liver subcellular membranes.* Biochim Biophys Acta, 1971. **249**(2): p. 462-92.
23. Osman, C., D.R. Voelker, and T. Langer, *Making heads or tails of phospholipids in mitochondria.* J Cell Biol. **192**(1): p. 7-16.
24. Daum, G., *Lipids of mitochondria.* Biochim Biophys Acta, 1985. **822**(1): p. 1-42.
25. Bretscher, M.S., *Membrane structure: some general principles.* Science, 1973. **181**(100): p. 622-9.
26. Daleke, D.L. and W.H. Huestis, *Erythrocyte morphology reflects the transbilayer distribution of incorporated phospholipids.* J Cell Biol, 1989. **108**(4): p. 1375-85.
27. Schroit, A.J., J.W. Madsen, and Y. Tanaka, *In vivo recognition and clearance of red blood cells containing phosphatidylserine in their plasma membranes.* J Biol Chem, 1985. **260**(8): p. 5131-8.
28. Bell, R.M., L.M. Ballas, and R.A. Coleman, *Lipid topogenesis.* J Lipid Res, 1981. **22**(3): p. 391-403.
29. Herrmann, A., A. Zachowski, and P.F. Devaux, *Protein-mediated phospholipid translocation in the endoplasmic reticulum with a low lipid specificity.* Biochemistry, 1990. **29**(8): p. 2023-7.
30. Bishop, W.R. and R.M. Bell, *Assembly of the endoplasmic reticulum phospholipid bilayer: the phosphatidylcholine transporter.* Cell, 1985. **42**(1): p. 51-60.

31. Kawashima, Y. and R.M. Bell, *Assembly of the endoplasmic reticulum phospholipid bilayer. Transporters for phosphatidylcholine and metabolites.* J Biol Chem, 1987. **262**(34): p. 16495-502.
32. Zilversmit, D.B. and M.E. Hughes, *Extensive exchange of rat liver microsomal phospholipids.* Biochim Biophys Acta, 1977. **469**(1): p. 99-110.
33. Marx, U., et al., *Rapid flip-flop of phospholipids in endoplasmic reticulum membranes studied by a stopped-flow approach.* Biophys J, 2000. **78**(5): p. 2628-40.
34. Seigneuret, M. and P.F. Devaux, *ATP-dependent asymmetric distribution of spin-labeled phospholipids in the erythrocyte membrane: relation to shape changes.* Proc Natl Acad Sci U S A, 1984. **81**(12): p. 3751-5.
35. Zilversmit, D.B., *Lipid transfer proteins.* J Lipid Res, 1984. **25**(13): p. 1563-9.
36. Daleke, D.L., *Phospholipid flippases.* J Biol Chem, 2007. **282**(2): p. 821-5.
37. Pomorski, T., et al., *Transbilayer movement of fluorescent and spin-labeled phospholipids in the plasma membrane of human fibroblasts: a quantitative approach.* J Cell Sci, 1996. **109 (Pt 3)**: p. 687-98.
38. Daleke, D.L. and J.V. Lyles, *Identification and purification of aminophospholipid flippases.* Biochim Biophys Acta, 2000. **1486**(1): p. 108-27.
39. Pomorski, T., et al., *Protein-mediated inward translocation of phospholipids occurs in both the apical and basolateral plasma membrane domains of epithelial cells.* Biochemistry, 1999. **38**(1): p. 142-50.
40. Martin, O.C. and R.E. Pagano, *Transbilayer movement of fluorescent analogs of phosphatidylserine and phosphatidylethanolamine at the plasma membrane of cultured cells. Evidence for a protein-mediated and ATP-dependent process(es).* J Biol Chem, 1987. **262**(12): p. 5890-8.
41. Sleight, R.G. and R.E. Pagano, *Transbilayer movement of a fluorescent phosphatidylethanolamine analogue across the plasma membranes of cultured mammalian cells.* J Biol Chem, 1985. **260**(2): p. 1146-54.
42. Muller, K., et al., *Protein-dependent translocation of aminophospholipids and asymmetric transbilayer distribution of phospholipids in the plasma membrane of ram sperm cells.* Biochemistry, 1994. **33**(33): p. 9968-74.
43. Muller, P., et al., *Transverse movement of spin-labeled phospholipids in the plasma membrane of a hepatocytic cell line (HepG2): implications for biliary lipid secretion.* Hepatology, 1996. **24**(6): p. 1497-503.
44. Devaux, P.F., et al., *How lipid flippases can modulate membrane structure.* Biochim Biophys Acta, 2008. **1778**(7-8): p. 1591-600.
45. Bitbol, M., et al., *Ion regulation of phosphatidylserine and phosphatidylethanolamine outside-inside translocation in human erythrocytes.* Biochim Biophys Acta, 1987. **904**(2): p. 268-82.
46. Bevers, E.M., et al., *Lipid translocation across the plasma membrane of mammalian cells.* Biochim Biophys Acta, 1999. **1439**(3): p. 317-30.
47. Anzai, K., Y. Yoshioka, and Y. Kirino, *Novel radioactive phospholipid probes as a tool for measurement of phospholipid translocation across biomembranes.* Biochim Biophys Acta, 1993. **1151**(1): p. 69-75.
48. Connor, J., et al., *Bidirectional transbilayer movement of phospholipid analogs in human red blood cells. Evidence for an ATP-dependent and protein-mediated process.* J Biol Chem, 1992. **267**(27): p. 19412-7.
49. Callaghan, R., R.C. Ford, and I.D. Kerr, *The translocation mechanism of P-glycoprotein.* FEBS Lett, 2006. **580**(4): p. 1056-63.
50. van Helvoort, A., et al., *MDR1 P-glycoprotein is a lipid translocase of broad specificity, while MDR3 P-glycoprotein specifically translocates phosphatidylcholine.* Cell, 1996. **87**(3): p. 507-17.
51. Borst, P. and R.O. Elferink, *Mammalian ABC transporters in health and disease.* Annu Rev Biochem, 2002. **71**: p. 537-92.
52. Hamon, Y., et al., *ABC1 promotes engulfment of apoptotic cells and transbilayer redistribution of phosphatidylserine.* Nat Cell Biol, 2000. **2**(7): p. 399-406.
53. Hamon, Y., O. Chambenoit, and G. Chimini, *ABCA1 and the engulfment of apoptotic cells.* Biochim Biophys Acta, 2002. **1585**(2-3): p. 64-71.
54. Emoto, K., et al., *Exposure of phosphatidylethanolamine on the surface of apoptotic cells.* Exp Cell Res, 1997. **232**(2): p. 430-4.
55. Williamson, P., et al., *Phospholipid scramblase activation pathways in lymphocytes.* Biochemistry, 2001. **40**(27): p. 8065-72.
56. Dean, M., Y. Hamon, and G. Chimini, *The human ATP-binding cassette (ABC) transporter superfamily.* J Lipid Res, 2001. **42**(7): p. 1007-17.
57. Bodzioch, M., et al., *The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease.* Nat Genet, 1999. **22**(4): p. 347-51.

58. Brooks-Wilson, A., et al., *Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency.* Nat Genet, 1999. **22**(4): p. 336-45.
59. Beharry, S., M. Zhong, and R.S. Molday, *N-retinylidene-phosphatidylethanolamine is the preferred retinoid substrate for the photoreceptor-specific ABC transporter ABCA4 (ABCR).* J Biol Chem, 2004. **279**(52): p. 53972-9.
60. de Vree, J.M., et al., *Mutations in the MDR3 gene cause progressive familial intrahepatic cholestasis.* Proc Natl Acad Sci U S A, 1998. **95**(1): p. 282-7.
61. Frasch, S.C., et al., *Phospholipid flip-flop and phospholipid scramblase 1 (PLSCR1) co-localize to uropod rafts in formylated Met-Leu-Phe-stimulated neutrophils.* J Biol Chem, 2004. **279**(17): p. 17625-33.
62. Sun, J., et al., *Plasma membrane phospholipid scramblase 1 is enriched in lipid rafts and interacts with the epidermal growth factor receptor.* Biochemistry, 2002. **41**(20): p. 6338-45.
63. Voelker, D.R., *Genetic and biochemical analysis of non-vesicular lipid traffic.* Annu Rev Biochem, 2009. **78**: p. 827-56.
64. Basse, F., et al., *Isolation of an erythrocyte membrane protein that mediates Ca2+-dependent transbilayer movement of phospholipid.* J Biol Chem, 1996. **271**(29): p. 17205-10.
65. Comfurius, P., et al., *Reconstitution of phospholipid scramblase activity from human blood platelets.* Biochemistry, 1996. **35**(24): p. 7631-4.
66. Somerharju, P., et al., *The superlattice model of lateral organization of membranes and its implications on membrane lipid homeostasis.* Biochim Biophys Acta, 2009. **1788**(1): p. 12-23.
67. Sprong, H., P. van der Sluijs, and G. van Meer, *How proteins move lipids and lipids move proteins.* Nat Rev Mol Cell Biol, 2001. **2**(7): p. 504-13.
68. Voelker, D.R., *Organelle biogenesis and intracellular lipid transport in eukaryotes.* Microbiol Rev, 1991. **55**(4): p. 543-60.
69. Jones, J.D. and T.E. Thompson, *Spontaneous phosphatidylcholine transfer by collision between vesicles at high lipid concentration.* Biochemistry, 1989. **28**(1): p. 129-34.
70. Mesmin, B. and F.R. Maxfield, *Intracellular sterol dynamics.* Biochim Biophys Acta, 2009. **1791**(7): p. 636-45.
71. Lev, S., *Non-vesicular lipid transport by lipid-transfer proteins and beyond.* Nat Rev Mol Cell Biol. **11**(10): p. 739-50.
72. Li, H., et al., *Both isoforms of mammalian phosphatidylinositol transfer protein are capable of binding and transporting sphingomyelin.* Biochim Biophys Acta, 2002. **1580**(1): p. 67-76.
73. Cheng, Z.J., et al., *Membrane microdomains, caveolae, and caveolar endocytosis of sphingolipids.* Mol Membr Biol, 2006. **23**(1): p. 101-10.
74. Cheng, Z.J., et al., *Distinct mechanisms of clathrin-independent endocytosis have unique sphingolipid requirements.* Mol Biol Cell, 2006. **17**(7): p. 3197-210.
75. Sleight, R.G. and R.E. Pagano, *Transport of a fluorescent phosphatidylcholine analog from the plasma membrane to the Golgi apparatus.* J Cell Biol, 1984. **99**(2): p. 742-51.
76. Lipsky, N.G. and R.E. Pagano, *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**(1): p. 27-34.
77. Kobayashi, T. and R.E. Pagano, *Lipid transport during mitosis. Alternative pathways for delivery of newly synthesized lipids to the cell surface.* J Biol Chem, 1989. **264**(10): p. 5966-73.
78. Kaplan, M.R. and R.D. Simoni, *Intracellular transport of phosphatidylcholine to the plasma membrane.* J Cell Biol, 1985. **101**(2): p. 441-5.
79. Sleight, R.G. and R.E. Pagano, *Rapid appearance of newly synthesized phosphatidylethanolamine at the plasma membrane.* J Biol Chem, 1983. **258**(15): p. 9050-8.
80. Vance, J.E., E.J. Aasman, and R. Szarka, *Brefeldin A does not inhibit the movement of phosphatidylethanolamine from its sites for synthesis to the cell surface.* J Biol Chem, 1991. **266**(13): p. 8241-7.
81. Voelker, D.R., *Reconstitution of phosphatidylserine import into rat liver mitochondria.* J Biol Chem, 1989. **264**(14): p. 8019-25.
82. Voelker, D.R., *Phosphatidylserine translocation to the mitochondrion is an ATP-dependent process in permeabilized animal cells.* Proc Natl Acad Sci U S A, 1989. **86**(24): p. 9921-5.
83. Voelker, D.R., *Characterization of phosphatidylserine synthesis and translocation in permeabilized animal cells.* J Biol Chem, 1990. **265**(24): p. 14340-6.
84. Holthuis, J.C. and T.P. Levine, *Lipid traffic: floppy drives and a superhighway.* Nat Rev Mol Cell Biol, 2005. **6**(3): p. 209-20.
85. Levine, T., *Short-range intracellular trafficking of small molecules across endoplasmic reticulum junctions.* Trends Cell Biol, 2004. **14**(9): p. 483-90.

86. Tsuneoka, M., et al., *Nonspecific lipid transfer protein (sterol carrier protein-2) is located in rat liver peroxisomes.* J Biochem, 1988. **104**(4): p. 560-4.
87. Vance, J.E. and Y.J. Shiao, *Intracellular trafficking of phospholipids: import of phosphatidylserine into mitochondria.* Anticancer Res, 1996. **16**(3B): p. 1333-9.
88. van Heusden, G.P., et al., *Chinese hamster ovary cells deficient in peroxisomes lack the nonspecific lipid transfer protein (sterol carrier protein 2).* J Biol Chem, 1990. **265**(7): p. 4105-10.
89. Trotter, P.J. and D.R. Voelker, *Lipid transport processes in eukaryotic cells.* Biochim Biophys Acta, 1994. **1213**(3): p. 241-62.
90. Wirtz, K.W., *Phospholipid transfer proteins.* Annu Rev Biochem, 1991. **60**: p. 73-99.
91. De Matteis, M.A., A. Di Campli, and G. D'Angelo, *Lipid-transfer proteins in membrane trafficking at the Golgi complex.* Biochim Biophys Acta, 2007. **1771**(6): p. 761-8.
92. Hanada, K., et al., *CERT and intracellular trafficking of ceramide.* Biochim Biophys Acta, 2007. **1771**(6): p. 644-53.
93. Kawano, M., et al., *Efficient trafficking of ceramide from the endoplasmic reticulum to the Golgi apparatus requires a VAMP-associated protein-interacting FFAT motif of CERT.* J Biol Chem, 2006. **281**(40): p. 30279-88.
94. Massey, J.B., et al., *Measurement and prediction of the rates of spontaneous transfer of phospholipids between plasma lipoproteins.* Biochim Biophys Acta, 1984. **794**(2): p. 274-80.
95. McLean, L.R. and M.C. Phillips, *Mechanism of cholesterol and phosphatidylcholine exchange or transfer between unilamellar vesicles.* Biochemistry, 1981. **20**(10): p. 2893-900.
96. McLean, L.R. and M.C. Phillips, *Kinetics of phosphatidylcholine and lysophosphatidylcholine exchange between unilamellar vesicles.* Biochemistry, 1984. **23**(20): p. 4624-30.
97. Bai, J. and R.E. Pagano, *Measurement of spontaneous transfer and transbilayer movement of BODIPY-labeled lipids in lipid vesicles.* Biochemistry, 1997. **36**(29): p. 8840-8.
98. Steck, T.L., F.J. Kezdy, and Y. Lange, *An activation-collision mechanism for cholesterol transfer between membranes.* J Biol Chem, 1988. **263**(26): p. 13023-31.
99. Hanada, K., *Discovery of the molecular machinery CERT for endoplasmic reticulum-to-Golgi trafficking of ceramide.* Mol Cell Biochem, 2006. **286**(1-2): p. 23-31.
100. Perkins, G., et al., *Electron tomography of neuronal mitochondria: three-dimensional structure and organization of cristae and membrane contacts.* J Struct Biol, 1997. **119**(3): p. 260-72.
101. Sleight, R.G., *Intracellular lipid transport in eukaryotes.* Annu Rev Physiol, 1987. **49**: p. 193-208.
102. Lebiedzinska, M., et al., *Interactions between the endoplasmic reticulum, mitochondria, plasma membrane and other subcellular organelles.* Int J Biochem Cell Biol, 2009. **41**(10): p. 1805-16.
103. Anderie, I., I. Schulz, and A. Schmid, *Direct interaction between ER membrane-bound PTP1B and its plasma membrane-anchored targets.* Cell Signal, 2007. **19**(3): p. 582-92.
104. de Brito, O.M. and L. Scorrano, *Mitofusin 2 tethers endoplasmic reticulum to mitochondria.* Nature, 2008. **456**(7222): p. 605-10.
105. Giorgi, C., et al., *Structural and functional link between the mitochondrial network and the endoplasmic reticulum.* Int J Biochem Cell Biol, 2009. **41**(10): p. 1817-27.
106. Wu, M.M., et al., *Ca2+ store depletion causes STIM1 to accumulate in ER regions closely associated with the plasma membrane.* J Cell Biol, 2006. **174**(6): p. 803-13.
107. Gincel, D., H. Zaid, and V. Shoshan-Barmatz, *Calcium binding and translocation by the voltage-dependent anion channel: a possible regulatory mechanism in mitochondrial function.* Biochem J, 2001. **358**(Pt 1): p. 147-55.
108. Rapizzi, E., et al., *Recombinant expression of the voltage-dependent anion channel enhances the transfer of Ca2+ microdomains to mitochondria.* J Cell Biol, 2002. **159**(4): p. 613-24.
109. Frischauf, I., et al., *The STIM/Orai coupling machinery.* Channels (Austin), 2008. **2**(4): p. 261-8.
110. Csordas, G., et al., *Structural and functional features and significance of the physical linkage between ER and mitochondria.* J Cell Biol, 2006. **174**(7): p. 915-21.
111. Shiao, Y.J., B. Balcerzak, and J.E. Vance, *A mitochondrial membrane protein is required for translocation of phosphatidylserine from mitochondria-associated membranes to mitochondria.* Biochem J, 1998. **331 (Pt 1)**: p. 217-23.
112. Vance, J.E., *Phospholipid synthesis in a membrane fraction associated with mitochondria.* J Biol Chem, 1990. **265**(13): p. 7248-56.
113. Vance, J.E., S.J. Stone, and J.R. Faust, *Abnormalities in mitochondria-associated membranes and phospholipid biosynthetic enzymes in the mnd/mnd mouse model of neuronal ceroid lipofuscinosis.* Biochim Biophys Acta, 1997. **1344**(3): p. 286-99.
114. Ladinsky, M.S., et al., *Golgi structure in three dimensions: functional insights from the normal rat kidney cell.* J Cell Biol, 1999. **144**(6): p. 1135-49.

115. Mogelsvang, S., et al., *Predicting function from structure: 3D structure studies of the mammalian Golgi complex.* Traffic, 2004. **5**(5): p. 338-45.
116. Kornmann, B. and P. Walter, *ERMES-mediated ER-mitochondria contacts: molecular hubs for the regulation of mitochondrial biology.* J Cell Sci. **123**(Pt 9): p. 1389-93.
117. Cerqua, C., et al., *Trichoplein/mitostatin regulates endoplasmic reticulum-mitochondria juxtaposition.* EMBO Rep. **11**(11): p. 854-60.
118. Nishizuka, Y., *Studies and perspectives of protein kinase C.* Science, 1986. **233**(4761): p. 305-12.
119. Arispe, N., et al., *Hsc70 and Hsp70 interact with phosphatidylserine on the surface of PC12 cells resulting in a decrease of viability.* Faseb J, 2004. **18**(14): p. 1636-45.
120. Ghosh, S., et al., *The cysteine-rich region of raf-1 kinase contains zinc, translocates to liposomes, and is adjacent to a segment that binds GTP-ras.* J Biol Chem, 1994. **269**(13): p. 10000-7.
121. Nagai, Y., et al., *An alternative splicing form of phosphatidylserine-specific phospholipase A1 that exhibits lysophosphatidylserine-specific lysophospholipase activity in humans.* J Biol Chem, 1999. **274**(16): p. 11053-9.
122. Nishizuka, Y., *Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C.* Science, 1992. **258**(5082): p. 607-14.
123. Yeung, T., et al., *Membrane phosphatidylserine regulates surface charge and protein localization.* Science, 2008. **319**(5860): p. 210-3.
124. Wang, X. and P.J. Quinn, *Cubic phase is induced by cholesterol in the dispersion of 1-palmitoyl-2-oleoyl-phosphatidylethanolamine.* Biochim Biophys Acta, 2002. **1564**(1): p. 66-72.
125. Jin, X.H., et al., *Discovery and characterization of a Ca2+-independent phosphatidylethanolamine N-acyltransferase generating the anandamide precursor and its congeners.* J Biol Chem, 2007. **282**(6): p. 3614-23.
126. Bratton, D.L., et al., *Appearance of phosphatidylserine on apoptotic cells requires calcium-mediated nonspecific flip-flop and is enhanced by loss of the aminophospholipid translocase.* J Biol Chem, 1997. **272**(42): p. 26159-65.
127. Fadok, V.A., et al., *Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages.* J Immunol, 1992. **148**(7): p. 2207-16.
128. Martin, S.J., et al., *Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl.* J Exp Med, 1995. **182**(5): p. 1545-56.
129. Li, M.O., et al., *Phosphatidylserine receptor is required for clearance of apoptotic cells.* Science, 2003. **302**(5650): p. 1560-3.
130. Miyanishi, M., et al., *Identification of Tim4 as a phosphatidylserine receptor.* Nature, 2007. **450**(7168): p. 435-9.
131. Park, D., et al., *BAI1 is an engulfment receptor for apoptotic cells upstream of the ELMO/Dock180/Rac module.* Nature, 2007. **450**(7168): p. 430-4.
132. Williamson, P. and R.A. Schlegel, *Hide and seek: the secret identity of the phosphatidylserine receptor.* J Biol, 2004. **3**(4): p. 14.
133. Frasch, S.C., et al., *Regulation of phospholipid scramblase activity during apoptosis and cell activation by protein kinase Cdelta.* J Biol Chem, 2000. **275**(30): p. 23065-73.
134. Aussel, C., C. Pelassy, and J.P. Breittmayer, *CD95 (Fas/APO-1) induces an increased phosphatidylserine synthesis that precedes its externalization during programmed cell death.* FEBS Lett, 1998. **431**(2): p. 195-9.
135. Stone, S.J. and J.E. Vance, *Phosphatidylserine synthase-1 and -2 are localized to mitochondria-associated membranes.* J Biol Chem, 2000. **275**(44): p. 34534-40.
136. Yu, A., et al., *Preferential externalization of newly synthesized phosphatidylserine in apoptotic U937 cells is dependent on caspase-mediated pathways.* Biochim Biophys Acta, 2000. **1487**(2-3): p. 296-308.
137. Grandmaison, P.A., T.S. Nanowski, and J.E. Vance, *Externalization of phosphatidylserine during apoptosis does not specifically require either isoform of phosphatidylserine synthase.* Biochim Biophys Acta, 2004. **1636**(1): p. 1-11.
138. Yu, A., et al., *Stimulation of phosphatidylserine biosynthesis and facilitation of UV-induced apoptosis in Chinese hamster ovary cells overexpressing phospholipid scramblase 1.* J Biol Chem, 2003. **278**(11): p. 9706-14.
139. Bevers, E.M., et al., *Generation of prothrombin-converting activity and the exposure of phosphatidylserine at the outer surface of platelets.* Eur J Biochem, 1982. **122**(2): p. 429-36.
140. Zwaal, R.F., P. Comfurius, and E.M. Bevers, *Scott syndrome, a bleeding disorder caused by defective scrambling of membrane phospholipids.* Biochim Biophys Acta, 2004. **1636**(2-3): p. 119-28.
141. Zwaal, R.F., P. Comfurius, and E.M. Bevers, *Lipid-protein interactions in blood coagulation.* Biochim Biophys Acta, 1998. **1376**(3): p. 433-53.

142. Sims, P.J. and T. Wiedmer, *Unraveling the mysteries of phospholipid scrambling.* Thromb Haemost, 2001. **86**(1): p. 266-75.
143. Thiagarajan, P. and J.F. Tait, *Collagen-induced exposure of anionic phospholipid in platelets and platelet-derived microparticles.* J Biol Chem, 1991. **266**(36): p. 24302-7.
144. Shaw, A.W., et al., *The local phospholipid environment modulates the activation of blood clotting.* J Biol Chem, 2007. **282**(9): p. 6556-63.
145. Lentz, B.R., *Exposure of platelet membrane phosphatidylserine regulates blood coagulation.* Prog Lipid Res, 2003. **42**(5): p. 423-38.
146. Flesch, F.M., et al., *Bicarbonate stimulated phospholipid scrambling induces cholesterol redistribution and enables cholesterol depletion in the sperm plasma membrane.* J Cell Sci, 2001. **114**(Pt 19): p. 3543-55.
147. Gadella, B.M. and R.A. Harrison, *The capacitating agent bicarbonate induces protein kinase A-dependent changes in phospholipid transbilayer behavior in the sperm plasma membrane.* Development, 2000. **127**(11): p. 2407-20.
148. Wang, L., C. Beserra, and D.L. Garbers, *A novel aminophospholipid transporter exclusively expressed in spermatozoa is required for membrane lipid asymmetry and normal fertilization.* Dev Biol, 2004. **267**(1): p. 203-15.
149. Rosing, J., et al., *Impaired factor X and prothrombin activation associated with decreased phospholipid exposure in platelets from a patient with a bleeding disorder.* Blood, 1985. **65**(6): p. 1557-61.
150. Bevers, E.M., R.F. Zwaal, and G.M. Willems, *The effect of phospholipids on the formation of immune complexes between autoantibodies and beta2-glycoprotein I or prothrombin.* Clin Immunol, 2004. **112**(2): p. 150-60.
151. McNeil, H.P., et al., *Anti-phospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation: beta 2-glycoprotein I (apolipoprotein H).* Proc Natl Acad Sci U S A, 1990. **87**(11): p. 4120-4.
152. Zwaal, R.F., P. Comfurius, and E.M. Bevers, *Surface exposure of phosphatidylserine in pathological cells.* Cell Mol Life Sci, 2005. **62**(9): p. 971-88.
153. de Jong, K., et al., *Characterization of the phosphatidylserine-exposing subpopulation of sickle cells.* Blood, 2001. **98**(3): p. 860-7.
154. Kuypers, F.A., et al., *Detection of altered membrane phospholipid asymmetry in subpopulations of human red blood cells using fluorescently labeled annexin V.* Blood, 1996. **87**(3): p. 1179-87.
155. Wood, B.L., D.F. Gibson, and J.F. Tait, *Increased erythrocyte phosphatidylserine exposure in sickle cell disease: flow-cytometric measurement and clinical associations.* Blood, 1996. **88**(5): p. 1873-80.
156. Eldor, A. and E.A. Rachmilewitz, *The hypercoagulable state in thalassemia.* Blood, 2002. **99**(1): p. 36-43.
157. Bonomini, M., et al., *Increased erythrocyte phosphatidylserine exposure in chronic renal failure.* J Am Soc Nephrol, 1999. **10**(9): p. 1982-90.
158. Cao, L.C., et al., *Oxalate-induced redistribution of phosphatidylserine in renal epithelial cells: implications for kidney stone disease.* Am J Nephrol, 2001. **21**(1): p. 69-77.
159. Wiessner, J.H., et al., *Mechanisms of calcium oxalate crystal attachment to injured renal collecting duct cells.* Kidney Int, 2001. **59**(2): p. 637-44.
160. Wahid, S.T., S.M. Marshall, and T.H. Thomas, *Increased platelet and erythrocyte external cell membrane phosphatidylserine in type 1 diabetes and microalbuminuria.* Diabetes Care, 2001. **24**(11): p. 2001-3.
161. Shiratsuchi, A., et al., *Phosphatidylserine-mediated phagocytosis of influenza A virus-infected cells by mouse peritoneal macrophages.* J Virol, 2000. **74**(19): p. 9240-4.
162. Kuo, C.C., et al., *Demonstration of Chlamydia pneumoniae in atherosclerotic lesions of coronary arteries.* J Infect Dis, 1993. **167**(4): p. 841-9.
163. Ran, S., A. Downes, and P.E. Thorpe, *Increased exposure of anionic phospholipids on the surface of tumor blood vessels.* Cancer Res, 2002. **62**(21): p. 6132-40.
164. Utsugi, T., et al., *Elevated expression of phosphatidylserine in the outer membrane leaflet of human tumor cells and recognition by activated human blood monocytes.* Cancer Res, 1991. **51**(11): p. 3062-6.
165. Kuge, O. and M. Nishijima, *Phosphatidylserine synthase I and II of mammalian cells.* Biochim Biophys Acta, 1997. **1348**(1-2): p. 151-6.
166. Vance, J.E. and D.E. Vance, *Does rat liver Golgi have the capacity to synthesize phospholipids for lipoprotein secretion?* J Biol Chem, 1988. **263**(12): p. 5898-909.
167. Voelker, D.R., *The ATP-dependent translocation of phosphatidylserine to the mitochondria is a process that is restricted to the autologous organelle.* J Biol Chem, 1993. **268**(10): p. 7069-74.
168. Voelker, D.R., *Phosphatidylserine functions as the major precursor of phosphatidylethanolamine in cultured BHK-21 cells.* Proc Natl Acad Sci U S A, 1984. **81**(9): p. 2669-73.

169. Kuge, O., M. Nishijima, and Y. Akamatsu, *A Chinese hamster cDNA encoding a protein essential for phosphatidylserine synthase I activity.* J Biol Chem, 1991. **266**(35): p. 24184-9.
170. Saito, K., M. Nishijima, and O. Kuge, *Genetic evidence that phosphatidylserine synthase II catalyzes the conversion of phosphatidylethanolamine to phosphatidylserine in Chinese hamster ovary cells.* J Biol Chem, 1998. **273**(27): p. 17199-205.
171. Kuge, O., M. Nishijima, and Y. Akamatsu, *Isolation of a somatic-cell mutant defective in phosphatidylserine biosynthesis.* Proc Natl Acad Sci U S A, 1985. **82**(7): p. 1926-30.
172. Voelker, D.R. and J.L. Frazier, *Isolation and characterization of a Chinese hamster ovary cell line requiring ethanolamine or phosphatidylserine for growth and exhibiting defective phosphatidylserine synthase activity.* J Biol Chem, 1986. **261**(3): p. 1002-8.
173. Sturbois-Balcerzak, B., et al., *Structure and expression of the murine phosphatidylserine synthase-1 gene.* J Biol Chem, 2001. **276**(11): p. 8205-12.
174. Bergo, M.O., et al., *Defining the importance of phosphatidylserine synthase 2 in mice.* J Biol Chem, 2002. **277**(49): p. 47701-8.
175. Stone, S.J. and J.E. Vance, *Cloning and expression of murine liver phosphatidylserine synthase (PSS)-2: differential regulation of phospholipid metabolism by PSS1 and PSS2.* Biochem J, 1999. **342 (Pt 1)**: p. 57-64.
176. Arikketh, D., R. Nelson, and J.E. Vance, *Defining the importance of phosphatidylserine synthase-1 (PSS1): unexpected viability of PSS1-deficient mice.* J Biol Chem, 2008. **283**(19): p. 12888-97.
177. Nishijima, M., O. Kuge, and Y. Akamatsu, *Phosphatidylserine biosynthesis in cultured Chinese hamster ovary cells. I. Inhibition of de novo phosphatidylserine biosynthesis by exogenous phosphatidylserine and its efficient incorporation.* J Biol Chem, 1986. **261**(13): p. 5784-9.
178. Kuge, O., K. Saito, and M. Nishijima, *Cloning of a Chinese hamster ovary (CHO) cDNA encoding phosphatidylserine synthase (PSS) II, overexpression of which suppresses the phosphatidylserine biosynthetic defect of a PSS I-lacking mutant of CHO-K1 cells.* J Biol Chem, 1997. **272**(31): p. 19133-9.
179. Kanfer, J.N., D. McCartney, and H. Hattori, *Regulation of the choline, ethanolamine and serine base exchange enzyme activities of rat brain microsomes by phosphorylation and dephosphorylation.* FEBS Lett, 1988. **240**(1-2): p. 101-4.
180. Kuge, O., et al., *Purification and characterization of Chinese hamster phosphatidylserine synthase 2.* J Biol Chem, 2003. **278**(43): p. 42692-8.
181. Kuge, O., K. Saito, and M. Nishijima, *Control of phosphatidylserine synthase II activity in Chinese hamster ovary cells.* J Biol Chem, 1999. **274**(34): p. 23844-9.
182. Hasegawa, K., et al., *Isolation and characterization of a Chinese hamster ovary cell mutant with altered regulation of phosphatidylserine biosynthesis.* J Biol Chem, 1989. **264**(33): p. 19887-92.
183. Kuge, O., et al., *Control of phosphatidylserine biosynthesis through phosphatidylserine-mediated inhibition of phosphatidylserine synthase I in Chinese hamster ovary cells.* Proc Natl Acad Sci U S A, 1998. **95**(8): p. 4199-203.
184. Stone, S.J., Z. Cui, and J.E. Vance, *Cloning and expression of mouse liver phosphatidylserine synthase-1 cDNA. Overexpression in rat hepatoma cells inhibits the CDP-ethanolamine pathway for phosphatidylethanolamine biosynthesis.* J Biol Chem, 1998. **273**(13): p. 7293-302.
185. Steenbergen, R., et al., *Phospholipid homeostasis in phosphatidylserine synthase-2-deficient mice.* Biochim Biophys Acta, 2006. **1761**(3): p. 313-23.
186. Kennedy, E.P. and S.B. Weiss, *The function of cytidine coenzymes in the biosynthesis of phospholipides.* J Biol Chem, 1956. **222**(1): p. 193-214.
187. Zhou, J. and J.D. Saba, *Identification of the first mammalian sphingosine phosphate lyase gene and its functional expression in yeast.* Biochem Biophys Res Commun, 1998. **242**(3): p. 502-7.
188. Lykidis, A., et al., *Overexpression of a mammalian ethanolamine-specific kinase accelerates the CDP-ethanolamine pathway.* J Biol Chem, 2001. **276**(3): p. 2174-9.
189. Vance, J.E., *Eukaryotic lipid-biosynthetic enzymes: the same but not the same.* Trends Biochem Sci, 1998. **23**(11): p. 423-8.
190. Henneberry, A.L. and C.R. McMaster, *Cloning and expression of a human choline/ethanolaminephosphotransferase: synthesis of phosphatidylcholine and phosphatidylethanolamine.* Biochem J, 1999. **339 (Pt 2)**: p. 291-8.
191. Henneberry, A.L., G. Wistow, and C.R. McMaster, *Cloning, genomic organization, and characterization of a human cholinephosphotransferase.* J Biol Chem, 2000. **275**(38): p. 29808-15.
192. Henneberry, A.L., M.M. Wright, and C.R. McMaster, *The major sites of cellular phospholipid synthesis and molecular determinants of Fatty Acid and lipid head group specificity.* Mol Biol Cell, 2002. **13**(9): p. 3148-61.
193. Kanfer, J. and E.P. Kennedy, *Metabolism and Function of Bacterial Lipids. Ii. Biosynthesis of Phospholipids in Escherichia Coli.* J Biol Chem, 1964. **239**: p. 1720-6.

194. Kuge, O., M. Nishijima, and Y. Akamatsu, *Phosphatidylserine biosynthesis in cultured Chinese hamster ovary cells. II. Isolation and characterization of phosphatidylserine auxotrophs.* J Biol Chem, 1986. **261**(13): p. 5790-4.
195. Miller, M.A. and C. Kent, *Characterization of the pathways for phosphatidylethanolamine biosynthesis in Chinese hamster ovary mutant and parental cell lines.* J Biol Chem, 1986. **261**(21): p. 9753-61.
196. Sundler, R. and B. Akesson, *Biosynthesis of phosphatidylethanolamines and phosphatidylcholines from ethanolamine and choline in rat liver.* Biochem J, 1975. **146**(2): p. 309-15.
197. Bleijerveld, O.B., et al., *The CDP-ethanolamine pathway and phosphatidylserine decarboxylation generate different phosphatidylethanolamine molecular species.* J Biol Chem, 2007. **282**(39): p. 28362-72.
198. Jasinska, R., J. Zborowski, and P. Somerharju, *Intramitochondrial distribution and transport of phosphatidylserine and its decarboxylation product, phosphatidylethanolamine. Application of pyrene-labeled species.* Biochim Biophys Acta, 1993. **1152**(1): p. 161-70.
199. Kevala, J.H. and H.Y. Kim, *Determination of substrate preference in phosphatidylserine decarboxylation by liquid chromatography-electrospray ionization mass spectrometry.* Anal Biochem, 2001. **292**(1): p. 130-8.
200. Voelker, D.R., *Phosphatidylserine decarboxylase.* Biochim Biophys Acta, 1997. **1348**(1-2): p. 236-44.
201. van Golde, L.M., et al., *Biosynthesis of lipids in Golgi complex and other subcellular fractions from rat liver.* Biochim Biophys Acta, 1974. **360**(2): p. 179-92.
202. Zborowski, J., A. Dygas, and L. Wojtczak, *Phosphatidylserine decarboxylase is located on the external side of the inner mitochondrial membrane.* FEBS Lett, 1983. **157**(1): p. 179-82.
203. Kuge, O., M. Nishijima, and Y. Akamatsu, *A cloned gene encoding phosphatidylserine decarboxylase complements the phosphatidylserine biosynthetic defect of a Chinese hamster ovary cell mutant.* J Biol Chem, 1991. **266**(10): p. 6370-6.
204. Kuge, O., et al., *Post-translational processing of the phosphatidylserine decarboxylase gene product in Chinese hamster ovary cells.* Biochem J, 1996. **319 (Pt 1)**: p. 33-8.
205. Schatz, G., *The protein import system of mitochondria.* J Biol Chem, 1996. **271**(50): p. 31763-6.
206. Nakashima, A., K. Hosaka, and J. Nikawa, *Cloning of a human cDNA for CTP-phosphoethanolamine cytidylyltransferase by complementation in vivo of a yeast mutant.* J Biol Chem, 1997. **272**(14): p. 9567-72.
207. Tian, Y., et al., *Placental thrombosis and spontaneous fetal death in mice deficient in ethanolamine kinase 2.* J Biol Chem, 2006. **281**(38): p. 28438-49.
208. Shiao, Y.J., G. Lupo, and J.E. Vance, *Evidence that phosphatidylserine is imported into mitochondria via a mitochondria-associated membrane and that the majority of mitochondrial phosphatidylethanolamine is derived from decarboxylation of phosphatidylserine.* J Biol Chem, 1995. **270**(19): p. 11190-8.
209. Steenbergen, R., et al., *Disruption of the phosphatidylserine decarboxylase gene in mice causes embryonic lethality and mitochondrial defects.* J Biol Chem, 2005. **280**(48): p. 40032-40.
210. Ardail, D., F. Lerme, and P. Louisot, *Involvement of contact sites in phosphatidylserine import into liver mitochondria.* J Biol Chem, 1991. **266**(13): p. 7978-81.
211. McArthur, M.J., et al., *Cellular uptake and intracellular trafficking of long chain fatty acids.* J Lipid Res, 1999. **40**(8): p. 1371-83.
212. Lands, W.E., *Metabolism of glycerolipides; a comparison of lecithin and triglyceride synthesis.* J Biol Chem, 1958. **231**(2): p. 883-8.
213. Six, D.A. and E.A. Dennis, *The expanding superfamily of phospholipase A(2) enzymes: classification and characterization.* Biochim Biophys Acta, 2000. **1488**(1-2): p. 1-19.
214. Waku, K. and Y. Nakazawa, *Acyltransferae activity to 1-acyl-, 1-O-alkenyl-, and 1-O-alkyl-glycero-3-phosphorylcholine in Ehrlich ascites tumor cells.* J Biochem, 1972. **72**(2): p. 495-7.
215. Balgoma, D., et al., *Lipidomic approaches to the study of phospholipase A2-regulated phospholipid fatty acid incorporation and remodeling.* Biochimie. **92**(6): p. 645-50.
216. Balsinde, J., M.A. Balboa, and E.A. Dennis, *Antisense inhibition of group VI Ca2+-independent phospholipase A2 blocks phospholipid fatty acid remodeling in murine P388D1 macrophages.* J Biol Chem, 1997. **272**(46): p. 29317-21.
217. Balsinde, J., et al., *Differential regulation of phospholipase D and phospholipase A2 by protein kinase C in P388D1 macrophages.* Biochem J, 1997. **321 (Pt 3)**: p. 805-9.
218. Balsinde, J., et al., *Inhibition of calcium-independent phospholipase A2 prevents arachidonic acid incorporation and phospholipid remodeling in P388D1 macrophages.* Proc Natl Acad Sci U S A, 1995. **92**(18): p. 8527-31.
219. Aoki, J., et al., *Structure and function of phosphatidylserine-specific phospholipase A1.* Biochim Biophys Acta, 2002. **1582**(1-3): p. 26-32.

220. Higgs, H.N., et al., *Cloning of a phosphatidic acid-preferring phospholipase A1 from bovine testis.* J Biol Chem, 1998. **273**(10): p. 5468-77.
221. Sato, T., et al., *Serine phospholipid-specific phospholipase A that is secreted from activated platelets. A new member of the lipase family.* J Biol Chem, 1997. **272**(4): p. 2192-8.
222. Burke, J.E. and E.A. Dennis, *Phospholipase A2 structure/function, mechanism, and signaling.* J Lipid Res, 2009. **50 Suppl**: p. S237-42.
223. Schaloske, R.H. and E.A. Dennis, *The phospholipase A2 superfamily and its group numbering system.* Biochim Biophys Acta, 2006. **1761**(11): p. 1246-59.
224. Singer, A.G., et al., *Interfacial kinetic and binding properties of the complete set of human and mouse groups I, II, V, X, and XII secreted phospholipases A2.* J Biol Chem, 2002. **277**(50): p. 48535-49.
225. Evans, J.H. and C.C. Leslie, *The cytosolic phospholipase A2 catalytic domain modulates association and residence time at Golgi membranes.* J Biol Chem, 2004. **279**(7): p. 6005-16.
226. Evans, J.H., et al., *Intracellular calcium signals regulating cytosolic phospholipase A2 translocation to internal membranes.* J Biol Chem, 2001. **276**(32): p. 30150-60.
227. Peters-Golden, M., et al., *Translocation of cytosolic phospholipase A2 to the nuclear envelope elicits topographically localized phospholipid hydrolysis.* Biochem J, 1996. **318 (Pt 3)**: p. 797-803.
228. Ohto, T., et al., *Identification of novel cytosolic phospholipase A(2)s, murine cPLA(2){delta}, {epsilon}, and {zeta}, which form a gene cluster with cPLA(2){beta}.* J Biol Chem, 2005. **280**(26): p. 24576-83.
229. Pickard, R.T., et al., *Molecular cloning of two new human paralogs of 85-kDa cytosolic phospholipase A2.* J Biol Chem, 1999. **274**(13): p. 8823-31.
230. Song, C., et al., *Molecular characterization of cytosolic phospholipase A2-beta.* J Biol Chem, 1999. **274**(24): p. 17063-7.
231. Underwood, K.W., et al., *A novel calcium-independent phospholipase A2, cPLA2-gamma, that is prenylated and contains homology to cPLA2.* J Biol Chem, 1998. **273**(34): p. 21926-32.
232. Leslie, C.C., *Regulation of arachidonic acid availability for eicosanoid production.* Biochem Cell Biol, 2004. **82**(1): p. 1-17.
233. Leslie, C.C., *Regulation of the specific release of arachidonic acid by cytosolic phospholipase A2.* Prostaglandins Leukot Essent Fatty Acids, 2004. **70**(4): p. 373-6.
234. Leslie, C.C., *Kinetic properties of a high molecular mass arachidonoyl-hydrolyzing phospholipase A2 that exhibits lysophospholipase activity.* J Biol Chem, 1991. **266**(17): p. 11366-71.
235. Channon, J.Y. and C.C. Leslie, *A calcium-dependent mechanism for associating a soluble arachidonoyl-hydrolyzing phospholipase A2 with membrane in the macrophage cell line RAW 264.7.* J Biol Chem, 1990. **265**(10): p. 5409-13.
236. Nalefski, E.A., et al., *Independent folding and ligand specificity of the C2 calcium-dependent lipid binding domain of cytosolic phospholipase A2.* J Biol Chem, 1998. **273**(3): p. 1365-72.
237. Nalefski, E.A., M.M. Slazas, and J.J. Falke, *Ca2+-signaling cycle of a membrane-docking C2 domain.* Biochemistry, 1997. **36**(40): p. 12011-8.
238. Perisic, O., et al., *Mapping the phospholipid-binding surface and translocation determinants of the C2 domain from cytosolic phospholipase A2.* J Biol Chem, 1999. **274**(21): p. 14979-87.
239. Schievella, A.R., et al., *Calcium-mediated translocation of cytosolic phospholipase A2 to the nuclear envelope and endoplasmic reticulum.* J Biol Chem, 1995. **270**(51): p. 30749-54.
240. Stewart, A., et al., *Enzymatic properties of human cytosolic phospholipase A(2)gamma.* J Biol Chem, 2002. **277**(33): p. 29526-36.
241. Lei, X., et al., *Calcium-independent phospholipase A2 (iPLA2 beta)-mediated ceramide generation plays a key role in the cross-talk between the endoplasmic reticulum (ER) and mitochondria during ER stress-induced insulin-secreting cell apoptosis.* J Biol Chem, 2008. **283**(50): p. 34819-32.
242. Cummings, B.S., J. McHowat, and R.G. Schnellmann, *Role of an endoplasmic reticulum Ca(2+)-independent phospholipase A(2) in oxidant-induced renal cell death.* Am J Physiol Renal Physiol, 2002. **283**(3): p. F492-8.
243. Mancuso, D.J., C.M. Jenkins, and R.W. Gross, *The genomic organization, complete mRNA sequence, cloning, and expression of a novel human intracellular membrane-associated calcium-independent phospholipase A(2).* J Biol Chem, 2000. **275**(14): p. 9937-45.
244. Akiba, S. and T. Sato, *Cellular function of calcium-independent phospholipase A2.* Biol Pharm Bull, 2004. **27**(8): p. 1174-8.
245. Balsinde, J. and M.A. Balboa, *Cellular regulation and proposed biological functions of group VIA calcium-independent phospholipase A2 in activated cells.* Cell Signal, 2005. **17**(9): p. 1052-62.
246. Balsinde, J., R. Perez, and M.A. Balboa, *Calcium-independent phospholipase A2 and apoptosis.* Biochim Biophys Acta, 2006. **1761**(11): p. 1344-50.

247. Perez, R., et al., *Role of group VIA calcium-independent phospholipase A2 in arachidonic acid release, phospholipid fatty acid incorporation, and apoptosis in U937 cells responding to hydrogen peroxide.* J Biol Chem, 2004. **279**(39): p. 40385-91.
248. Ackermann, E.J., E.S. Kempner, and E.A. Dennis, *Ca(2+)-independent cytosolic phospholipase A2 from macrophage-like P388D1 cells. Isolation and characterization.* J Biol Chem, 1994. **269**(12): p. 9227-33.
249. Balboa, M.A., et al., *Identity between the Ca2+-independent phospholipase A2 enzymes from P388D1 macrophages and Chinese hamster ovary cells.* J Biol Chem, 1997. **272**(13): p. 8576-80.
250. Ma, Z., et al., *Pancreatic islets express a Ca2+-independent phospholipase A2 enzyme that contains a repeated structural motif homologous to the integral membrane protein binding domain of ankyrin.* J Biol Chem, 1997. **272**(17): p. 11118-27.
251. Winstead, M.V., J. Balsinde, and E.A. Dennis, *Calcium-independent phospholipase A(2): structure and function.* Biochim Biophys Acta, 2000. **1488**(1-2): p. 28-39.
252. Jenkins, C.M., et al., *Identification, cloning, expression, and purification of three novel human calcium-independent phospholipase A2 family members possessing triacylglycerol lipase and acylglycerol transacylase activities.* J Biol Chem, 2004. **279**(47): p. 48968-75.
253. van Tienhoven, M., et al., *Human neuropathy target esterase catalyzes hydrolysis of membrane lipids.* J Biol Chem, 2002. **277**(23): p. 20942-8.
254. Shen, Z., et al., *Phorbol 12-myristate 13-acetate stimulates lysophosphatidic acid secretion from ovarian and cervical cancer cells but not from breast or leukemia cells.* Gynecol Oncol, 1998. **71**(3): p. 364-8.
255. Ramanadham, S., et al., *Studies of the role of group VI phospholipase A2 in fatty acid incorporation, phospholipid remodeling, lysophosphatidylcholine generation, and secretagogue-induced arachidonic acid release in pancreatic islets and insulinoma cells.* J Biol Chem, 1999. **274**(20): p. 13915-27.
256. Ma, Z., et al., *Studies of insulin secretory responses and of arachidonic acid incorporation into phospholipids of stably transfected insulinoma cells that overexpress group VIA phospholipase A2 (iPLA2beta) indicate a signaling rather than a housekeeping role for iPLA2beta.* J Biol Chem, 2001. **276**(16): p. 13198-208.
257. Min, J.H., et al., *Membrane-bound plasma platelet activating factor acetylhydrolase acts on substrate in the aqueous phase.* Biochemistry, 1999. **38**(39): p. 12935-42.
258. Gelb, M.H., J.H. Min, and M.K. Jain, *Do membrane-bound enzymes access their substrates from the membrane or aqueous phase: interfacial versus non-interfacial enzymes.* Biochim Biophys Acta, 2000. **1488**(1-2): p. 20-7.
259. Abe, A. and J.A. Shayman, *Purification and characterization of 1-O-acylceramide synthase, a novel phospholipase A2 with transacylase activity.* J Biol Chem, 1998. **273**(14): p. 8467-74.
260. Hiraoka, M., A. Abe, and J.A. Shayman, *Cloning and characterization of a lysosomal phospholipase A2, 1-O-acylceramide synthase.* J Biol Chem, 2002. **277**(12): p. 10090-9.
261. Hishikawa, D., et al., *Discovery of a lysophospholipid acyltransferase family essential for membrane asymmetry and diversity*. Proc Natl Acad Sci U S A, 2008. **105**(8): p. 2830-5.
262. Zhao, Y., et al., *Identification and characterization of a major liver lysophosphatidylcholine acyltransferase.* J Biol Chem, 2008. **283**(13): p. 8258-65.
263. Chen, X., et al., *Identification and characterization of a lysophosphatidylcholine acyltransferase in alveolar type II cells.* Proc Natl Acad Sci U S A, 2006. **103**(31): p. 11724-9.
264. Harayama, T., et al., *Identification of a novel noninflammatory biosynthetic pathway of platelet-activating factor*. J Biol Chem, 2008. **283**(17): p. 11097-106.
265. Shindou, H., et al., *A single enzyme catalyzes both platelet-activating factor production and membrane biogenesis of inflammatory cells. Cloning and characterization of acetyl-CoA:LYSO-PAF acetyltransferase.* J Biol Chem, 2007. **282**(9): p. 6532-9.
266. Heikinheimo, L. and P. Somerharju, *Translocation of pyrene-labeled phosphatidylserine from the plasma membrane to mitochondria diminishes systematically with molecular hydrophobicity: implications on the maintenance of high phosphatidylserine content in the inner leaflet of the plasma membrane.* Biochim Biophys Acta, 2002. **1591**(1-3): p. 75-85.
267. Kuge, O., Y. Yamakawa, and M. Nishijima, *Enhancement of transport-dependent decarboxylation of phosphatidylserine by S100B protein in permeabilized Chinese hamster ovary cells.* J Biol Chem, 2001. **276**(26): p. 23700-6.
268. Voelker, D.R., *Disruption of phosphatidylserine translocation to the mitochondria in baby hamster kidney cells.* J Biol Chem, 1985. **260**(27): p. 14671-6.
269. Rizzuto, R., et al., *Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca2+ responses.* Science, 1998. **280**(5370): p. 1763-6.

270. Vance, J.E., *Newly made phosphatidylserine and phosphatidylethanolamine are preferentially translocated between rat liver mitochondria and endoplasmic reticulum.* J Biol Chem, 1991. **266**(1): p. 89-97.
271. Shore, G.C. and J.R. Tata, *Two fractions of rough endoplasmic reticulum from rat liver. I. Recovery of rapidly sedimenting endoplasmic reticulum in association with mitochondria.* J Cell Biol, 1977. **72**(3): p. 714-25.
272. Ardail, D., et al., *Involvement of mitochondrial contact sites in the subcellular compartmentalization of phospholipid biosynthetic enzymes.* J Biol Chem, 1993. **268**(34): p. 25985-92.
273. Voelker, D.R., *Adriamycin disrupts phosphatidylserine import into the mitochondria of permeabilized CHO-K1 cells.* J Biol Chem, 1991. **266**(19): p. 12185-8.
274. Hovius, R., et al., *On the mechanism of the mitochondrial decarboxylation of phosphatidylserine.* J Biol Chem, 1992. **267**(24): p. 16790-5.
275. Emoto, K., et al., *Isolation of a Chinese hamster ovary cell mutant defective in intramitochondrial transport of phosphatidylserine.* Proc Natl Acad Sci U S A, 1999. **96**(22): p. 12400-5.
276. Yaffe, M.P. and E.P. Kennedy, *Intracellular phospholipid movement and the role of phospholipid transfer proteins in animal cells.* Biochemistry, 1983. **22**(6): p. 1497-507.
277. Blok, M.C., K.W. Wirtz, and G.L. Scherphof, *Exchange of phospholipids between microsomes and inner and outer mitochondrial membranes of rat liver.* Biochim Biophys Acta, 1971. **233**(1): p. 61-75.
278. Daum, G. and J.E. Vance, *Import of lipids into mitochondria.* Prog Lipid Res, 1997. **36**(2-3): p. 103-30.
279. McMurray, W.C. and R.M. Dawson, *Phospholipid exchange reactions within the liver cell.* Biochem J, 1969. **112**(1): p. 91-108.
280. Camici, O. and L. Corazzi, *Import of phosphatidylethanolamine for the assembly of rat brain mitochondrial membranes.* J Membr Biol, 1995. **148**(2): p. 169-76.
281. Postle, A.D., et al., *Probing phospholipid dynamics by electrospray ionisation mass spectrometry.* Prog Lipid Res, 2007. **46**(3-4): p. 200-24.
282. Hunt, A.N. and A.D. Postle, *Mass spectrometry determination of endonuclear phospholipid composition and dynamics.* Methods, 2006. **39**(2): p. 104-11.
283. de Kroon, A.I., et al., *Continuous equilibration of phosphatidylcholine and its precursors between endoplasmic reticulum and mitochondria in yeast.* Mol Biol Cell, 2003. **14**(5): p. 2142-50.
284. DeLong, C.J., et al., *Molecular distinction of phosphatidylcholine synthesis between the CDP-choline pathway and phosphatidylethanolamine methylation pathway.* J Biol Chem, 1999. **274**(42): p. 29683-8.
285. Burdge, G.C., A.N. Hunt, and A.D. Postle, *Mechanisms of hepatic phosphatidylcholine synthesis in adult rat: effects of pregnancy.* Biochem J, 1994. **303 (Pt 3)**: p. 941-7.
286. Postle, A.D. and A.N. Hunt, *Dynamic lipidomics with stable isotope labelling.* J Chromatogr B Analyt Technol Biomed Life Sci, 2009. **877**(26): p. 2716-21.
287. Schmid, P.C., E. Deli, and H.H. Schmid, *Generation and remodeling of phospholipid molecular species in rat hepatocytes.* Arch Biochem Biophys, 1995. **319**(1): p. 168-76.
288. Koval, M. and R.E. Pagano, *Lipid recycling between the plasma membrane and intracellular compartments: transport and metabolism of fluorescent sphingomyelin analogues in cultured fibroblasts.* J Cell Biol, 1989. **108**(6): p. 2169-81.
289. Lipsky, N.G. and R.E. Pagano, *Sphingolipid metabolism in cultured fibroblasts: microscopic and biochemical studies employing a fluorescent ceramide analogue.* Proc Natl Acad Sci U S A, 1983. **80**(9): p. 2608-12.
290. Pagano, R.E., K.J. Longmuir, and O.C. Martin, *Intracellular translocation and metabolism of a fluorescent phosphatidic acid analogue in cultured fibroblasts.* J Biol Chem, 1983. **258**(3): p. 2034-40.
291. Ting, A.E. and R.E. Pagano, *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**(10): p. 5337-40.
292. Bitbol, M. and P.F. Devaux, *Measurement of outward translocation of phospholipids across human erythrocyte membrane.* Proc Natl Acad Sci U S A, 1988. **85**(18): p. 6783-7.
293. Pagano, R.E. and K.J. Longmuir, *Phosphorylation, transbilayer movement, and facilitated intracellular transport of diacylglycerol are involved in the uptake of a fluorescent analog of phosphatidic acid by cultured fibroblasts.* J Biol Chem, 1985. **260**(3): p. 1909-16.
294. Tanhuanpaa, K. and P. Somerharju, *gamma-cyclodextrins greatly enhance translocation of hydrophobic fluorescent phospholipids from vesicles to cells in culture. Importance of molecular hydrophobicity in phospholipid trafficking studies.* J Biol Chem, 1999. **274**(50): p. 35359-66.
295. Somerharju, P., *Pyrene-labeled lipids as tools in membrane biophysics and cell biology.* Chem Phys Lipids, 2002. **116**(1-2): p. 57-74.
296. Child, P., et al., *Acyl selectivity in the transfer of molecular species of phosphatidylcholines from human erythrocytes.* Biochim Biophys Acta, 1985. **812**(2): p. 321-32.

297. van Meer, G., et al., *Protein-stimulated exchange of phosphatidylcholine between intact erythrocytes and various membrane systems.* Biochim Biophys Acta, 1980. **598**(1): p. 173-7.
298. van Meer, G. and K. Simons, *An efficient method for introducing defined lipids into the plasma membrane of mammalian cells.* J Cell Biol, 1983. **97**(5 Pt 1): p. 1365-74.
299. Ohtani, Y., et al., *Differential effects of alpha-, beta- and gamma-cyclodextrins on human erythrocytes.* Eur J Biochem, 1989. **186**(1-2): p. 17-22.
300. Ohvo, H. and J.P. Slotte, *Cyclodextrin-mediated removal of sterols from monolayers: effects of sterol structure and phospholipids on desorption rate.* Biochemistry, 1996. **35**(24): p. 8018-24.
301. Zidovetzki, R. and I. Levitan, *Use of cyclodextrins to manipulate plasma membrane cholesterol content: evidence, misconceptions and control strategies.* Biochim Biophys Acta, 2007. **1768**(6): p. 1311-24.
302. Tanhuanpaa, K., et al., *Characteristics of pyrene phospholipid/gamma-cyclodextrin complex.* Biophys J, 2001. **81**(3): p. 1501-10.
303. Voelker, D.R., *Interorganelle transport of aminoglycerophospholipids.* Biochim Biophys Acta, 2000. **1486**(1): p. 97-107.
304. Griffiths, G., R. Back, and M. Marsh, *A quantitative analysis of the endocytic pathway in baby hamster kidney cells.* J Cell Biol, 1989. **109**(6 Pt 1): p. 2703-20.
305. Bozidis, P., C.D. Williamson, and A.M. Colberg-Poley, *Mitochondrial and secretory human cytomegalovirus UL37 proteins traffic into mitochondrion-associated membranes of human cells.* J Virol, 2008. **82**(6): p. 2715-26.
306. Wieckowski, M.R., et al., *Isolation of mitochondria-associated membranes and mitochondria from animal tissues and cells.* Nat Protoc, 2009. **4**(11): p. 1582-90.
307. Forner, F., E.A. Arriaga, and M. Mann, *Mild protease treatment as a small-scale biochemical method for mitochondria purification and proteomic mapping of cytoplasm-exposed mitochondrial proteins.* J Proteome Res, 2006. **5**(12): p. 3277-87.
308. Gilquin, B., et al., *The AAA+ ATPase ATAD3A controls mitochondrial dynamics at the interface of the inner and outer membranes.* Mol Cell Biol. **30**(8): p. 1984-96.
309. Hovius, R., et al., *Improved methods to isolate and subfractionate rat liver mitochondria. Lipid composition of the inner and outer membrane.* Biochim Biophys Acta, 1990. **1021**(2): p. 217-26.
310. Jelsema, C.L. and D.J. Morre, *Distribution of phospholipid biosynthetic enzymes among cell components of rat liver.* J Biol Chem, 1978. **253**(21): p. 7960-71.
311. Vance, J.E. and D.E. Vance, *Specific pools of phospholipids are used for lipoprotein secretion by cultured rat hepatocytes.* J Biol Chem, 1986. **261**(10): p. 4486-91.
312. Lucas, K.K. and E.A. Dennis, *Distinguishing phospholipase A2 types in biological samples by employing group-specific assays in the presence of inhibitors.* Prostaglandins Other Lipid Mediat, 2005. **77**(1-4): p. 235-48.
313. Yang, H.C., et al., *Group-specific assays that distinguish between the four major types of mammalian phospholipase A2.* Anal Biochem, 1999. **269**(2): p. 278-88.
314. De Petrocellis, L., et al., *Novel inhibitors of brain, neuronal, and basophilic anandamide amidohydrolase.* Biochem Biophys Res Commun, 1997. **231**(1): p. 82-8.
315. Kell, P.J., et al., *Inhibition of platelet-activating factor (PAF) acetylhydrolase by methyl arachidonyl fluorophosphonate potentiates PAF synthesis in thrombin-stimulated human coronary artery endothelial cells.* J Pharmacol Exp Ther, 2003. **307**(3): p. 1163-70.
316. Riendeau, D., et al., *Arachidonyl trifluoromethyl ketone, a potent inhibitor of 85-kDa phospholipase A2, blocks production of arachidonate and 12-hydroxyeicosatetraenoic acid by calcium ionophore-challenged platelets.* J Biol Chem, 1994. **269**(22): p. 15619-24.
317. Balboa, M.A., R. Perez, and J. Balsinde, *Amplification mechanisms of inflammation: paracrine stimulation of arachidonic acid mobilization by secreted phospholipase A2 is regulated by cytosolic phospholipase A2-derived hydroperoxyeicosatetraenoic acid.* J Immunol, 2003. **171**(2): p. 989-94.
318. Ono, T., et al., *Characterization of a novel inhibitor of cytosolic phospholipase A2alpha, pyrrophenone.* Biochem J, 2002. **363**(Pt 3): p. 727-35.
319. Jenkins, C.M., et al., *Identification of calcium-independent phospholipase A2 (iPLA2) beta, and not iPLA2gamma, as the mediator of arginine vasopressin-induced arachidonic acid release in A-10 smooth muscle cells. Enantioselective mechanism-based discrimination of mammalian iPLA2s.* J Biol Chem, 2002. **277**(36): p. 32807-14.
320. Balsinde, J. and E.A. Dennis, *Bromoenol lactone inhibits magnesium-dependent phosphatidate phosphohydrolase and blocks triacylglycerol biosynthesis in mouse P388D1 macrophages.* J Biol Chem, 1996. **271**(50): p. 31937-41.
321. Johnson, C.A., et al., *Regulation of cyclooxygenase-2 expression by phosphatidate phosphohydrolase in human amnionic WISH cells.* J Biol Chem, 1999. **274**(39): p. 27689-93.

322. Bonin, F., et al., *Anti-apoptotic actions of the platelet-activating factor acetylhydrolase I alpha2 catalytic subunit.* J Biol Chem, 2004. **279**(50): p. 52425-36.
323. Masuda, S., et al., *Neuronal expression and neuritogenic action of group X secreted phospholipase A2.* J Biol Chem, 2005. **280**(24): p. 23203-14.
324. Murakami, M., et al., *Cellular distribution, post-translational modification, and tumorigenic potential of human group III secreted phospholipase A(2).* J Biol Chem, 2005. **280**(26): p. 24987-98.
325. Pawliczak, R., et al., *Cytosolic phospholipase A2 Group IValpha but not secreted phospholipase A2 Group IIA, V, or X induces interleukin-8 and cyclooxygenase-2 gene and protein expression through peroxisome proliferator-activated receptors gamma 1 and 2 in human lung cells.* J Biol Chem, 2004. **279**(47): p. 48550-61.
326. Su, X., et al., *Small interfering RNA knockdown of calcium-independent phospholipases A2 beta or gamma inhibits the hormone-induced differentiation of 3T3-L1 preadipocytes.* J Biol Chem, 2004. **279**(21): p. 21740-8.
327. Bao, S., et al., *Male mice that do not express group VIA phospholipase A2 produce spermatozoa with impaired motility and have greatly reduced fertility.* J Biol Chem, 2004. **279**(37): p. 38194-200.
328. Ramanadham, S., et al., *Age-related changes in bone morphology are accelerated in group VIA phospholipase A2 (iPLA2beta)-null mice.* Am J Pathol, 2008. **172**(4): p. 868-81.
329. Balgoma, D., et al., *Calcium-independent phospholipase A2-mediated formation of 1,2-diarachidonoyl-glycerophosphoinositol in monocytes.* Febs J, 2008. **275**(24): p. 6180-91.
330. Heikinheimo, L. and P. Somerharju, *Translocation of phosphatidylthreonine and -serine to mitochondria diminishes exponentially with increasing molecular hydrophobicity.* Traffic, 2002. **3**(5): p. 367-77.
331. Peterson, B.L. and B.S. Cummings, *A review of chromatographic methods for the assessment of phospholipids in biological samples.* Biomed Chromatogr, 2006. **20**(3): p. 227-43.
332. Nakanishi, H., et al., *Analysis of oxidized phosphatidylcholines as markers for oxidative stress, using multiple reaction monitoring with theoretically expanded data sets with reversed-phase liquid chromatography/tandem mass spectrometry.* J Chromatogr B Analyt Technol Biomed Life Sci, 2009. **877**(13): p. 1366-74.
333. Haimi, P., et al., *Software tools for analysis of mass spectrometric lipidome data.* Anal Chem, 2006. **78**(24): p. 8324-31.
334. Hermansson, M., et al., *Automated quantitative analysis of complex lipidomes by liquid chromatography/mass spectrometry.* Anal Chem, 2005. **77**(7): p. 2166-75.
335. Katajamaa, M. and M. Oresic, *Processing methods for differential analysis of LC/MS profile data.* BMC Bioinformatics, 2005. **6**: p. 179.
336. Koivusalo, M., et al., *Quantitative determination of phospholipid compositions by ESI-MS: effects of acyl chain length, unsaturation, and lipid concentration on instrument response.* J Lipid Res, 2001. **42**(4): p. 663-72.
337. Han, X. and R.W. Gross, *Global analyses of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry: a bridge to lipidomics.* J Lipid Res, 2003. **44**(6): p. 1071-9.
338. Pulfer, M. and R.C. Murphy, *Electrospray mass spectrometry of phospholipids.* Mass Spectrom Rev, 2003. **22**(5): p. 332-64.
339. Han, X. and R.W. Gross, *Shotgun lipidomics: electrospray ionization mass spectrometric analysis and quantitation of cellular lipidomes directly from crude extracts of biological samples.* Mass Spectrom Rev, 2005. **24**(3): p. 367-412.
340. Han, X. and R.W. Gross, *Shotgun lipidomics: multidimensional MS analysis of cellular lipidomes.* Expert Rev Proteomics, 2005. **2**(2): p. 253-64.
341. Cui, Z. and M.J. Thomas, *Phospholipid profiling by tandem mass spectrometry.* J Chromatogr B Analyt Technol Biomed Life Sci, 2009. **877**(26): p. 2709-15.
342. Griffiths, W.J., et al., *Electrospray and tandem mass spectrometry in biochemistry.* Biochem J, 2001. **355**(Pt 3): p. 545-61.
343. Brugger, B., et al., *Quantitative analysis of biological membrane lipids at the low picomole level by nano-electrospray ionization tandem mass spectrometry.* Proc Natl Acad Sci U S A, 1997. **94**(6): p. 2339-44.
344. Lehmann, W.D., et al., *Characterization and quantification of rat bile phosphatidylcholine by electrospray-tandem mass spectrometry.* Anal Biochem, 1997. **246**(1): p. 102-10.
345. Yetukuri, L., et al., *Informatics and computational strategies for the study of lipids.* Mol Biosyst, 2008. **4**(2): p. 121-7.

346. Sullards, M.C., et al., *Structure-specific, quantitative methods for analysis of sphingolipids by liquid chromatography-tandem mass spectrometry: "inside-out" sphingolipidomics.* Methods Enzymol, 2007. **432**: p. 83-115.
347. DeLong, C.J., et al., *Molecular species composition of rat liver phospholipids by ESI-MS/MS: the effect of chromatography.* J Lipid Res, 2001. **42**(12): p. 1959-68.
348. DeLong, C.J., A.M. Hicks, and Z. Cui, *Disruption of choline methyl group donation for phosphatidylethanolamine methylation in hepatocarcinoma cells.* J Biol Chem, 2002. **277**(19): p. 17217-25.
349. Kakela, R., P. Somerharju, and J. Tyynela, *Analysis of phospholipid molecular species in brains from patients with infantile and juvenile neuronal-ceroid lipofuscinosis using liquid chromatography-electrospray ionization mass spectrometry.* J Neurochem, 2003. **84**(5): p. 1051-65.
350. Patel, K.M., J.D. Morrisett, and J.T. Sparrow, *A convenient synthesis of phosphatidylcholines: acylation of sn-glycero-3-phosphocholine with fatty acid anhydride and 4-pyrrolidinopyridine.* J Lipid Res, 1979. **20**(5): p. 674-7.
351. Silversand, C. and C. Haux, *Improved high-performance liquid chromatographic method for the separation and quantification of lipid classes: application to fish lipids.* J Chromatogr B Biomed Sci Appl, 1997. **703**(1-2): p. 7-14.
352. Bartlett, E.M. and D.H. Lewis, *Spectrophotometric determination of phosphate esters in the presence and absence of orthophosphate.* Anal Biochem, 1970. **36**(1): p. 159-67.
353. Folch, J., M. Lees, and G.H. Sloane Stanley, *A simple method for the isolation and purification of total lipides from animal tissues.* J Biol Chem, 1957. **226**(1): p. 497-509.
354. Heikinheimo, L. and P. Somerharju, *Preferential decarboxylation of hydrophilic phosphatidylserine species in cultured cells. Implications on the mechanism of transport to mitochondria and cellular aminophospholipid species compositions.* J Biol Chem, 1998. **273**(6): p. 3327-35.
355. Hsu, F.F. and J. Turk, *Charge-remote and charge-driven fragmentation processes in diacyl glycerophosphoethanolamine upon low-energy collisional activation: a mechanistic proposal.* J Am Soc Mass Spectrom, 2000. **11**(10): p. 892-9.
356. Hsu, F.F. and J. Turk, *Charge-driven fragmentation processes in diacyl glycerophosphatidic acids upon low-energy collisional activation. A mechanistic proposal.* J Am Soc Mass Spectrom, 2000. **11**(9): p. 797-803.
357. Storrie, B. and E.A. Madden, *Isolation of subcellular organelles.* Methods Enzymol, 1990. **182**: p. 203-25.
358. Lowry, O.H., et al., *Protein measurement with the Folin phenol reagent.* J Biol Chem, 1951. **193**(1): p. 265-75.
359. Gamble, W., et al., *Procedure for determination of free and total cholesterol in micro- or nanogram amounts suitable for studies with cultured cells.* J Lipid Res, 1978. **19**(8): p. 1068-70.
360. Fernandez-Vizarra, E., et al., *Isolation of mitochondria for biogenetical studies: An update.* Mitochondrion. **10**(3): p. 253-62.
361. Samborski, R.W., N.D. Ridgway, and D.E. Vance, *Evidence that only newly made phosphatidylethanolamine is methylated to phosphatidylcholine and that phosphatidylethanolamine is not significantly deacylated-reacylated in rat hepatocytes.* J Biol Chem, 1990. **265**(30): p. 18322-9.
362. Besterman, J.M. and P.L. Domanico, *Association and metabolism of exogenously-derived lysophosphatidylcholine by cultured mammalian cells: kinetics and mechanisms.* Biochemistry, 1992. **31**(7): p. 2046-56.
363. Deng, L., et al., *Incorporation and remodeling of phosphatidylethanolamine containing short acyl residues in yeast.* Biochim Biophys Acta. **1801**(6): p. 635-45.
364. Kol, M.A., et al., *Uptake and remodeling of exogenous phosphatidylethanolamine in E. coli.* Biochim Biophys Acta, 2004. **1636**(2-3): p. 205-12.
365. Tanaka, K., et al., *Incorporation and remodeling of extracellular phosphatidylcholine with short acyl residues in Saccharomyces cerevisiae.* Biochim Biophys Acta, 2008. **1781**(8): p. 391-9.
366. Fukasawa, M., et al., *Reduction of sphingomyelin level without accumulation of ceramide in Chinese hamster ovary cells affects detergent-resistant membrane domains and enhances cellular cholesterol efflux to methyl-beta -cyclodextrin.* J Biol Chem, 2000. **275**(44): p. 34028-34.
367. Kilsdonk, E.P., et al., *Cellular cholesterol efflux mediated by cyclodextrins.* J Biol Chem, 1995. **270**(29): p. 17250-6.
368. Choi, Y.A., et al., *Methyl-beta-cyclodextrin inhibits cell growth and cell cycle arrest via a prostaglandin E(2) independent pathway.* Exp Mol Med, 2004. **36**(1): p. 78-84.
369. Grosse, P.Y., F. Bressolle, and F. Pinguet, *Methyl-beta-cyclodextrin in HL-60 parental and multidrug-resistant cancer cell lines: effect on the cytotoxic activity and intracellular accumulation of doxorubicin.* Cancer Chemother Pharmacol, 1997. **40**(6): p. 489-94.

370. Hipler, U.C., et al., *Influence of cyclodextrins on the proliferation of HaCaT keratinocytes in vitro.* J Biomed Mater Res A, 2007. **83**(1): p. 70-9.
371. Tijburg, L.B., R.W. Samborski, and D.E. Vance, *Evidence that remodeling of the fatty acids of phosphatidylcholine is regulated in isolated rat hepatocytes and involves both the sn-1 and sn-2 positions.* Biochim Biophys Acta, 1991. **1085**(2): p. 184-90.
372. Thompson, W. and H. Belina, *Rapid accumulation of diacyl lipid in rat liver microsomes by selective acylation of 2-acyl-sn-glycero-3-phosphorylserine.* Biochim Biophys Acta, 1986. **876**(3): p. 379-86.
373. MacDonald, J.I. and H. Sprecher, *Phospholipid fatty acid remodeling in mammalian cells.* Biochim Biophys Acta, 1991. **1084**(2): p. 105-21.
374. Nixon, A.B., D.G. Greene, and R.L. Wykle, *Comparison of acceptor and donor substrates in the CoA-independent transacylase reaction in human neutrophils.* Biochim Biophys Acta, 1996. **1300**(3): p. 187-96.
375. Maxfield, F.R. and M. Mondal, *Sterol and lipid trafficking in mammalian cells.* Biochem Soc Trans, 2006. **34**(Pt 3): p. 335-9.
376. Asai, K., et al., *Human group IVC phospholipase A2 (cPLA2gamma). Roles in the membrane remodeling and activation induced by oxidative stress.* J Biol Chem, 2003. **278**(10): p. 8809-14.
377. Murakami, M., et al., *Group VIB Ca2+-independent phospholipase A2gamma promotes cellular membrane hydrolysis and prostaglandin production in a manner distinct from other intracellular phospholipases A2.* J Biol Chem, 2005. **280**(14): p. 14028-41.
378. Rouzer, C.A., et al., *Lipid profiling reveals glycerophospholipid remodeling in zymosan-stimulated macrophages.* Biochemistry, 2007. **46**(20): p. 6026-42.
379. Kramer, R.M., C.R. Pritzker, and D. Deykin, *Coenzyme A-mediated arachidonic acid transacylation in human platelets.* J Biol Chem, 1984. **259**(4): p. 2403-6.
380. Reddy, P.V. and H.H. Schmid, *Acylation of dog heart lysophosphatidylserine by transacylase activity.* Biochim Biophys Acta, 1987. **922**(3): p. 379-85.
381. Chambers, K. and W.J. Brown, *Characterization of a novel CI-976-sensitive lysophospholipid acyltransferase that is associated with the Golgi complex.* Biochem Biophys Res Commun, 2004. **313**(3): p. 681-6.
382. Ghosh, M., et al., *Properties of the Group IV phospholipase A2 family.* Prog Lipid Res, 2006. **45**(6): p. 487-510.
383. Kinsey, G.R., et al., *Identification and distribution of endoplasmic reticulum iPLA2.* Biochem Biophys Res Commun, 2005. **327**(1): p. 287-93.
384. Seleznev, K., et al., *Calcium-independent phospholipase A2 localizes in and protects mitochondria during apoptotic induction by staurosporine.* J Biol Chem, 2006. **281**(31): p. 22275-88.
385. Williams, S.D. and R.A. Gottlieb, *Inhibition of mitochondrial calcium-independent phospholipase A2 (iPLA2) attenuates mitochondrial phospholipid loss and is cardioprotective.* Biochem J, 2002. **362**(Pt 1): p. 23-32.
386. Xu, Y., et al., *Remodeling of cardiolipin by phospholipid transacylation.* J Biol Chem, 2003. **278**(51): p. 51380-5.
387. Yang, Y., J. Cao, and Y. Shi, *Identification and characterization of a gene encoding human LPGAT1, an endoplasmic reticulum-associated lysophosphatidylglycerol acyltransferase.* J Biol Chem, 2004. **279**(53): p. 55866-74.
388. Ousley, A.H. and P. Morell, *Individual molecular species of phosphatidylcholine and phosphatidylethanolamine in myelin turn over at different rates.* J Biol Chem, 1992. **267**(15): p. 10362-9.
389. Vance, D.E., C.J. Walkey, and Z. Cui, *Phosphatidylethanolamine N-methyltransferase from liver.* Biochim Biophys Acta, 1997. **1348**(1-2): p. 142-50.