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Review

Regulation of membrane phospholipid biosynthesis in mammalian cells

Shin-ya Morita *, Yoshito Ikeda

Department of Pharmacy, Shiga University of Medical Science Hospital, Otsu, Shiga 520-2192, Japan

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ABSTRACT

In mammalian cells, phospholipids and cholesterol are assembled into bilayer membranes forming the plasma membrane, nuclear envelope, mitochondria, endoplasmic reticulum, Golgi apparatus, lysosomes, and endosomes. Phospholipids are divided into classes based on the molecular structures, including phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidic acid, phosphatidylinositol, phosphatidylglycerol, cardiolipin, and sphingomyelin. In addition to their structural roles, phospholipids play important roles in many cellular processes, such as membrane protein regulation, membrane trafficking, cell growth, apoptosis, and intracellular signaling. Thus, abnormal phospholipid metabolism is associated with various diseases. In mammalian cells, phospholipid classes are generated through several enzymatic steps, predominantly in the endoplasmic reticulum, mitochondria, and Golgi apparatus. In recent years, various enzymes involved in the biosynthesis of phospholipid classes have been identified. However, little is known about the regulatory mechanisms underlying the biosynthesis of phospholipid classes. Using our recently developed enzymatic fluorometric assays for all major phospholipid classes, we have demonstrated changes in phospholipid composition in intracellular organelles during cell growth. In this review, we summarize the current understanding of the properties and functions of phospholipid biosynthesis enzymes, and discuss their regulatory mechanisms.

1. Introduction

Phospholipids are amphiphilic molecules that consist of a hydrophilic head group and two hydrophobic acyl chains (Fig. 1). Phospholipids are divided into two groups: glycerophospholipids with glycerol backbones and sphingophospholipids with sphingosine backbones. On the basis of head group structures, glycerophospholipids are subdivided into classes, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidic acid (PA), phosphatidylinositol (PI), phosphatidylglycerol (PG), and cardiolipin (CL). The glycerol backbone is esterified with long-chain fatty acids at the *sn*-1 and *sn*-2 positions. Sphingomyelin (SM) is a sphingophospholipid class with a phosphocholine moiety in the head group. Lysophospholipids, such as lysophosphatidylcholine (LPC), lysophosphatidylethanolamine and lysophosphatidic acid (LPA), contain only one acyl chain. Ether-linked phospholipids, including plasmanylcholine, plasmenylcholine, plasmanylethanolamine, and plasmenylethanolamine, are characterized by an ether bond at the *sn*-1

Abbreviations: BMP, bis(monoacylglycero)phosphate; CCT, CTP:phosphocholine cytidylyltransferase; CDP, cytidine diphosphate; CDS, CDP-diacylglycerol synthase; CEPT, choline/ethanolaminephosphotransferase; CHO, Chinese hamster ovary; CK, choline kinase; CL, cardiolipin; CLS, cardiolipin synthase; CMP, cytidine monophosphate; CPT, CDP-choline:diacylglycerol cholinephosphotransferase; CTP, cytidine triphosphate; DG, diacylglycerol; DGK, diacylglycerol kinase; ECT, CTP: phosphoethanolamine cytidylyltransferase; EK, ethanolamine kinase; EPT, CDP-ethanolamine:diacylglycerol ethanolaminephosphotransferase; ER, endoplasmic reticulum; G3P, glycerol-3-phosphate; GPAT, glycerophosphate acyltransferase; LDL, low density lipoprotein; LPA, lysophosphatidic acid; LPAAT, lysophosphatidic acid acyltransferase; LPC, lysophosphatidylcholine; MAM, mitochondria-associated membrane; MARCKS, myristoylated alanine-rich C-kinase substrate; MitoPLD, mitochondria-specific phospholipase D; Nir2, *N*-terminal domain-interacting receptor 2; ORP, oxysterol-binding protein-related protein; PA, phosphatidic acid; PAP, phosphatidic acid phosphatase; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine *N*-methyltransferase; PG, phosphatidylglycerol; PGP, phosphatidylglycerophosphate; PGPP, phosphatidylglycerophosphate phosphatase; PGS, phosphatidylglycerophosphate synthase; PH, pleckstrin homology; PI, phosphatidylinositol; PIS, phosphatidylinositol synthase; PIP, phosphatidylinositol phosphate; PLC, phospholipase C; PLD, phospholipase D; PS, phosphatidylserine; PSD, phosphatidylserine decarboxylase; PSS, phosphatidylserine synthase; PTPMT, protein tyrosine phosphatase localized to the mitochondrion; SM, sphingomyelin; SMase, sphingomyelinase; SMS, sphingomyelin synthase; Sp1, specificity protein-1; TAMM, mitochondrial translocator assembly and maintenance homolog protein; XBP-1, X-box-binding protein-1.

* Corresponding author.
E-mail address: smorita@belle.shiga-med.ac.jp (S.-y. Morita).

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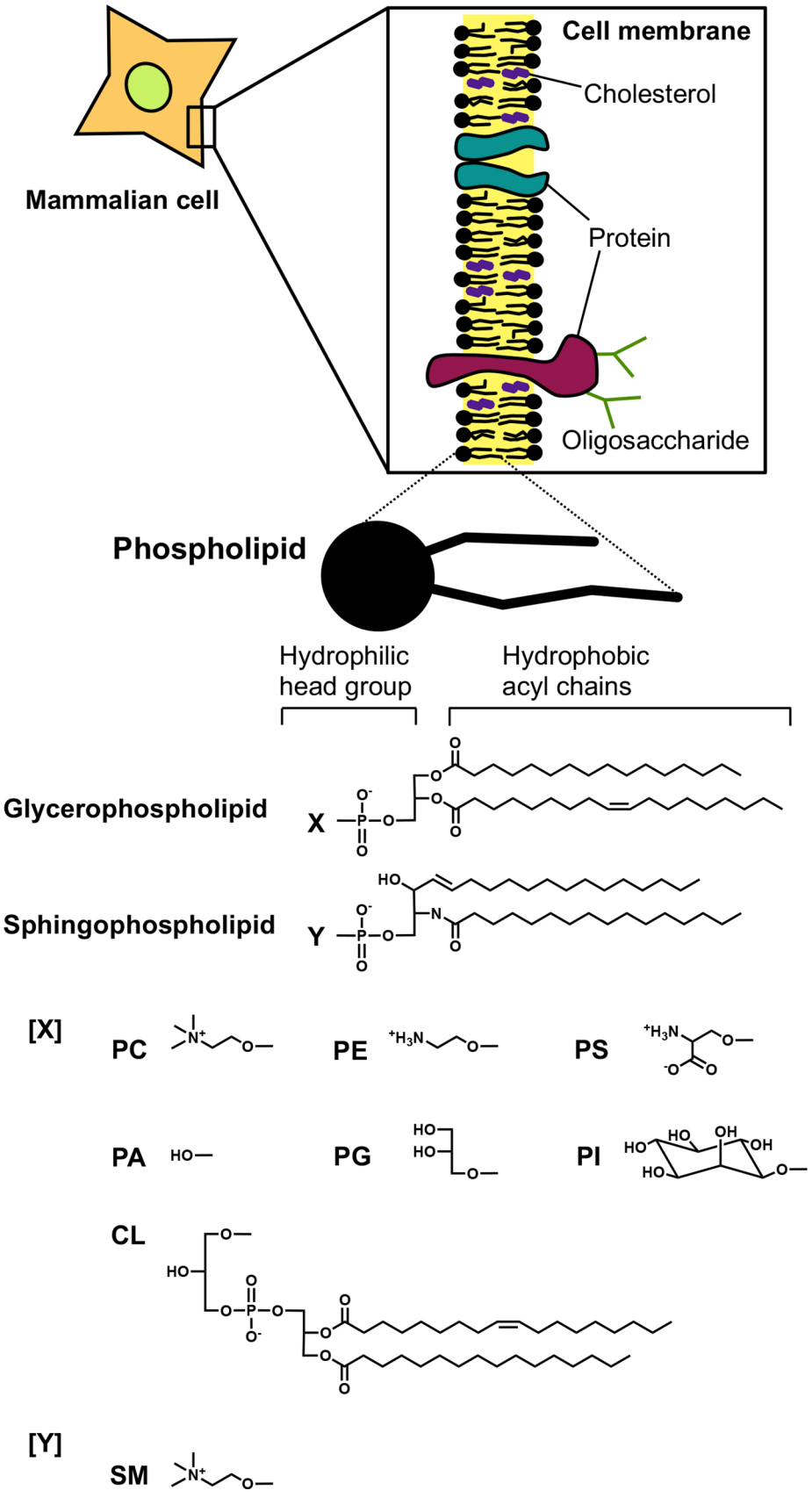

Fig. 1. Chemical structures of major phospholipid classes in mammalian cells. Phospholipids and cholesterol are spontaneously assembled into bilayer membranes in cells. Phospholipids consist of a hydrophilic head group and two hydrophobic acyl chains. There are two groups, glycerophospholipids with a glycerol backbone and sphingophospholipids with a sphingosine backbone. Glycerophospholipids are further divided based on head group structures into classes. CL, cardiolipin; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin.

position of the glycerol backbone (Fig. 2). Plasmenylcholine and plasmenylethanolamine, also known as plasmalogens, contain a *cis* double bond adjacent to the ether bond, forming a vinyl-ether linkage. Therefore, thousands of phospholipid molecular species exist in one cell.

In mammalian cells, phospholipids and cholesterol are essential components of bilayer membranes, forming the plasma membrane, nuclear envelope, mitochondria, endoplasmic reticulum (ER), Golgi apparatus, peroxisomes, lysosomes, and endosomes. The quantitatively major phospholipid classes in mammalian cell membranes are PC and PE, and PI, PS, PA, PG, and SM are minor components [1–4]. Phospholipids are asymmetrically distributed across the plasma membrane bilayer. PS and PE are enriched in the inner leaflet of the plasma membrane, whereas PC and SM are predominantly located on the outer leaflet. In plasma membranes, SM and cholesterol are highly enriched in lipid raft microdomains, where acyl chains are well ordered and tightly packed [5]. PC, which has a cylindrical shape, forms lipid bilayers, whereas cone-shaped lipids, such as PE, PA, and CL, form non-lamellar structures with negative curvature. PE and CL are abundant in the mitochondrial cristae, tube-like extensions of the inner mitochondrial membrane [6]. PA acts as a fusogenic lipid, lowering the activation energy for negative curvature, and participates in vesicular traffic [7]. PA generation is linked to the mitochondrial fusion [8].

Phospholipid monolayers envelop adipocyte lipid droplets or plasma lipoproteins, including very low-density lipoproteins secreted from hepatocytes and chylomicrons secreted from enterocytes [9,10]. During the conversion from very low-density lipoprotein to low-density lipoprotein (LDL), the SM/PC ratio increases at the surface monolayer, whereas the PE/PC ratio decreases [11]. LDL particles containing apolipoprotein B-100 are internalized by various cells through interactions with LDL receptors on the plasma membranes [10]. At the plasma membrane, high-density lipoproteins are produced from phospholipids and cholesterol by the ATP-binding cassette transporter ABCA1 and apolipoprotein A-I [10,12,13]. Extracellular vesicles, composed of phospholipid bilayer membranes, proteins, RNAs, and DNAs, are generated from endosomal compartments or plasma membranes [14]. The secretion of phospholipids, predominantly PC (>95 %), from hepatocytes into bile is mediated by the transporter ABCB4 on canalicular membranes [15,16]. In type II alveolar epithelial cells, ABCA3 plays an essential role in the formation of lamellar bodies and in the secretion of pulmonary surfactants, which contain PC (70 %-80 %), PG (<10 %), PI, PE, and PS [17–19].

In addition to their structural roles in membranes, phospholipids play important roles in various cellular processes, such as membrane trafficking, membrane protein localization and regulation, autophagy, cellular signaling, cell proliferation and differentiation, cell migration and apoptosis. Microtubule-associated protein 1 light chain 3 conjugated with PE triggers autophagy [20]. During apoptosis, PS is externalized on the outer leaflet of the plasma membrane by Xk-related protein 8 and then recognized by phagocytes [21]. In activated platelets, PS is exposed on the platelet surface by transmembrane protein 16F to trigger the blood clotting [22]. PS activates several proteins, such as Raf-1 protein kinase, protein kinase C, dynamin-1 and neutral sphingomyelinase (SMase) [23–26]. PA mediates the membrane localization and activation of protein kinase C, mammalian target of rapamycin, type I PI(4)P 5-kinase, Raf-1 kinase, sphingosine kinase 1, cAMP phosphodiesterase 4A1, phospholipase C, the tyrosine phosphatase SHP-1 and the guanine nucleotide exchange factor Son of sevenless [27–36]. PG is also a physiological activator of protein kinase C [37]. In the inner mitochondrial membrane, CL stabilizes the electron transfer complex and promotes ATP production [38]. CL in the outer leaflet of the inner mitochondrial membrane is tightly bound to cytochrome *c* [39]. CL also plays a crucial role in apoptosis by enabling docking and activation of pro-apoptotic Bcl-2 proteins [39]. In addition to PI, PI phosphates (PIPs) have key roles in numerous cellular functions [40,41].

Impaired phospholipid metabolism leads to diverse disorders, including dyslipidemia, atherosclerosis, liver diseases, respiratory diseases, autoimmune diseases, neurological diseases, cardiac and skeletal myopathies, and cancers [10,16,18,42,43]. In mammalian cells,

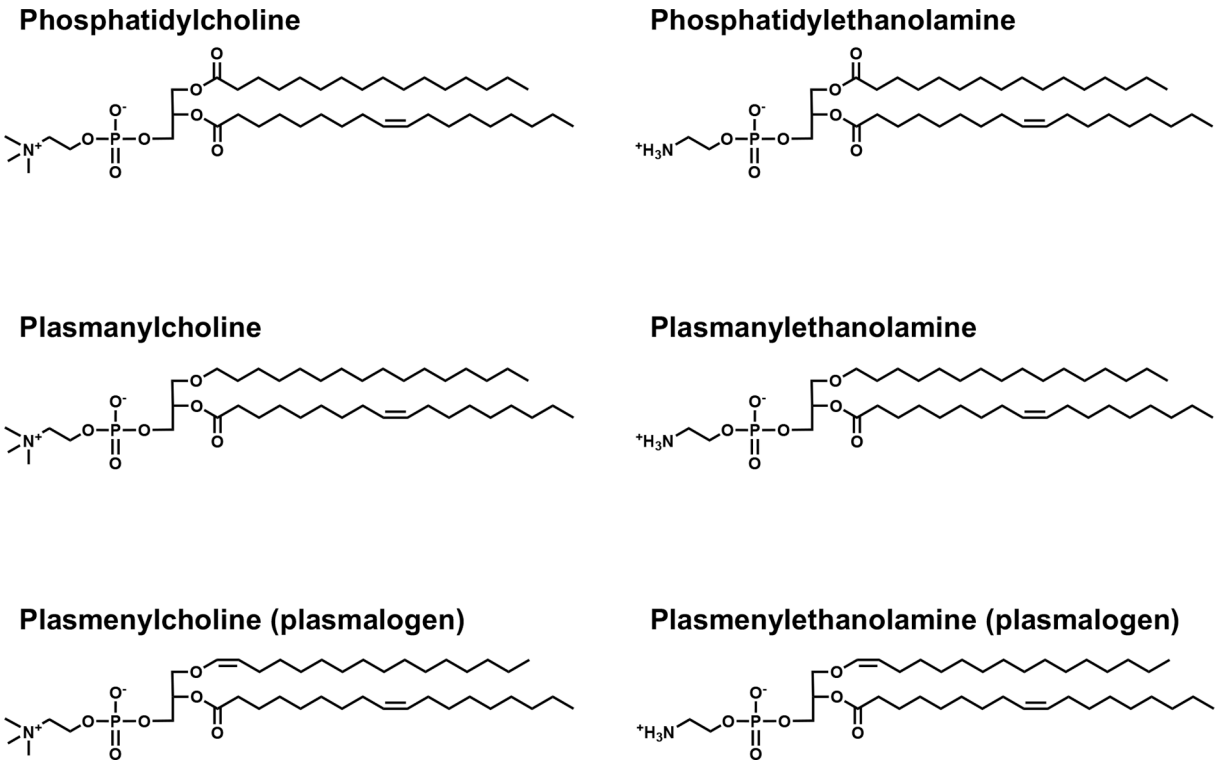

Fig. 2. Chemical structures of ether-linked phospholipids. Plasmanylcholine, plasmenylcholine, plasmanylethanolamine, or plasmenylethanolamine contains an ether bond, but not an ester bond, at the *sn*-1 position of the glycerol backbone. Plasmenylcholine or plasmenylethanolamine contains a *cis* double bond forming a vinyl-ether linkage. Plasmenylcholine and plasmenylethanolamine are also called plasmalogens.

phospholipid classes are synthesized mainly in the ER, mitochondria, and Golgi apparatus through the actions of many enzymes (Figs. 3 and 4). In this review, we describe the current knowledge of the characteristics and functions of enzymes related to the biosynthesis of phospholipid classes and their regulation in mammalian cells.

2. PC biosynthesis

PC is the most abundant phospholipid (40 %-60 % of total phospholipids) in mammalian cells and accounts for approximately 95 % of total choline pool [1]. All mammalian cells synthesize PC via the cytidine diphosphate (CDP)-choline pathway, also called the Kennedy pathway, which comprises choline kinase, cytidine triphosphate (CTP): phosphocholine cytidylyltransferase (CCT), and CDP-choline: diacylglycerol cholinephosphotransferase (CPT) [1,44,45]. In hepatocytes, PC is also synthesized by PE *N*-methyltransferase (PEMT) [1,46]. LPC is acylated to form PC by LPC acyltransferase activity [47]. PC is catabolized by phospholipase A_2, phospholipase C (PLC), phospholipase D (PLD), PS synthase (PSS), and SM synthase (SMS).

The CDP-choline pathway involves a three-step enzymatic process discovered by Kennedy et al. [48]. In the first step, choline is phosphorylated to phosphocholine by choline kinases CKα and CKβ using ATP (Table 1) [49,50]. Both choline kinase isoforms are soluble proteins present in the cytosol. In humans, the sequence of CKβ (395 amino acids) is 62.1 % similar to that of CKα (457 amino acids). These choline kinases are ubiquitously expressed and function as homodimers or heterodimers [51]. Both isoforms also use ethanolamine as a substrate [52]. The c-Src-dependent phosphorylation of human CKα at the Tyr-197 residue located proximal to the dimer interface and at the Tyr-333 residue within the choline-binding motif enhances catalytic activity [53]. Ser-39 and Ser-40 residues of human CKβ, not located in the catalytic domain, are phosphorylated by protein kinase A, which increases the enzymatic activity toward choline but not ethanolamine [54].

In the second step of the CDP-choline pathway, CCT catalyzes the conversion of phosphocholine and CTP into CDP-choline and pyrophosphate [1,44,45]. This step is irreversible, because pyrophosphate is rapidly hydrolyzed to inorganic phosphate in cells [55]. CTP, but not ATP, is the activating molecule for phospholipid biosynthesis. Both human isoforms, CCTα (367 amino acids) and CCTβ (369 amino acids), are activated upon reversible binding to membranes of the ER or nuclei [56,57]. CCTα, with a nuclear localization sequence in its *N*-terminus, is mainly in the nucleus and in the cytoplasm, whereas CCTβ, without a nuclear localization signal, is a cytoplasmic enzyme [57–59]. Although the soluble form of CCT is inactive, the membrane binding of CCT is mediated via a long amphipathic α-helical domain and releases the catalytic site [60,61]. Anionic phospholipids, fatty acids, and diacylglycerol (DG) promotes the membrane binding and activation of rat CCT, and the stimulation of rat CCT activity by anionic phospholipids is as follows: PI(4)P > CL > PA > PG ≈ PS > PI ≫ PC [62]. Human CCTα is highly phosphorylated at Ser residues in its C-terminal region [56,63].

In the final step, CPT catalyzes the transfer of phosphocholine from CDP-choline to DG to yield PC and cytidine monophosphate (CMP). The amino acid sequence of human CPT1 (406 amino acids) is 72.0 % similar to that of human choline/ethanolaminephosphotransferase 1 (CEPT1) (416 amino acids). Human CPT1 is specific for CDP-choline, whereas human CEPT1 uses CDP-choline and CDP-ethanolamine [64,65]. Human CPT1, with seven predicted membrane-spanning domains, is primarily localized in the Golgi apparatus [65,66]. Human CEPT1, with seven predicted membrane-spanning domains, is present in the ER and nuclear membranes [64,66].

In addition to the CDP-choline pathway, PEMT catalyzes the biosynthesis of PC via the sequential transfer of three methyl groups from *S*-adenosylmethionine to the amino head group of PE in the livers of mammals [1]. PEMT is localized in the ER, particularly in the ER subdomain called mitochondria-associated membrane (MAM) [67,68]. In rat primary hepatocytes, the PEMT-controlled pathway is responsible

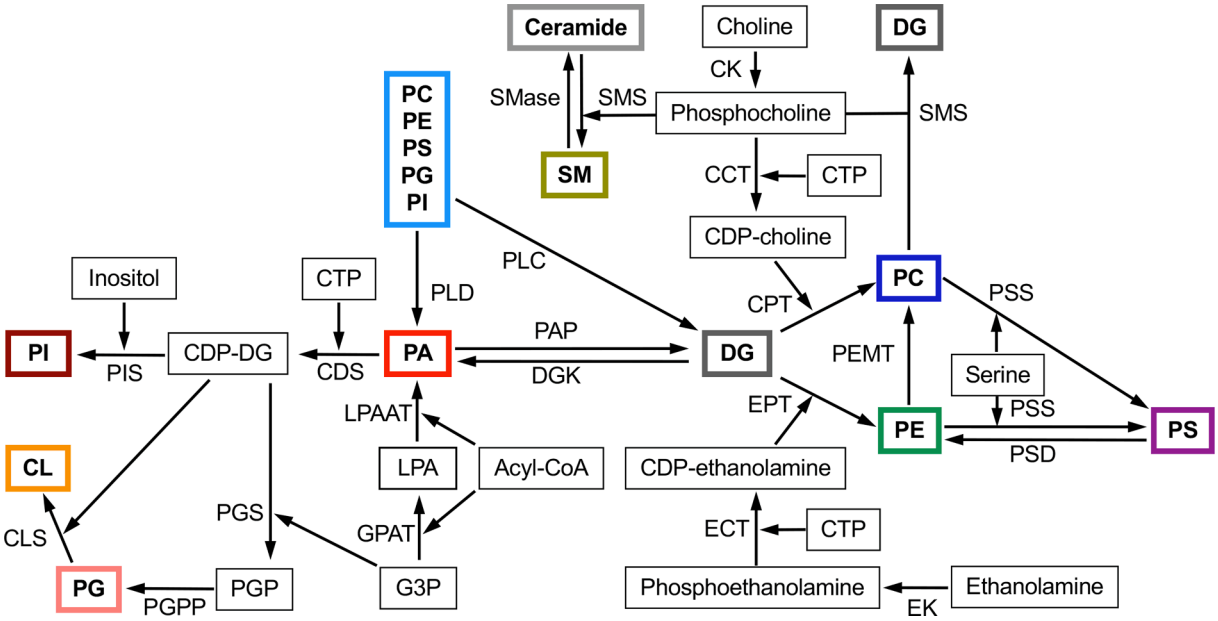

Fig. 3. Pathways for biosynthesis of major phospholipid classes in mammalian cells. CCT, CTP:phosphocholine cytidylyltransferase; CDP, cytidine diphosphate; CDS, CDP-diacylglycerol synthase; CK, choline kinase; CL, cardiolipin; CLS, cardiolipin synthase; CPT, CDP-choline:diacylglycerol cholinephosphotransferase; CTP, cytidine triphosphate; DG, diacylglycerol; DGK, diacylglycerol kinase; ECT, CTP:phosphoethanolamine cytidylyltransferase; EK, ethanolamine kinase; EPT, CDP-ethanolamine:diacylglycerol ethanolaminephosphotransferase; G3P, glycerol-3-phosphate; GPAT, glycerophosphate acyltransferase; LPA, lysophosphatidic acid; LPAAT, lysophosphatidic acid acyltransferase; PA, phosphatidic acid; PAP, phosphatidic acid phosphatase; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine *N*-methyltransferase; PG, phosphatidylglycerol; PGP, phosphatidylglycerophosphate; PGPP, phosphatidylglycerophosphate phosphatase; PGS, phosphatidylglycerophosphate synthase; PI, phosphatidylinositol; PIS, phosphatidylinositol synthase; PLC, phospholipase C; PLD, phospholipase D; PS, phosphatidylserine; PSD, phosphatidylserine decarboxylase; PSS, phosphatidylserine synthase; SM, sphingomyelin; SMase, sphingomyelinase; SMS, sphingomyelin synthase.

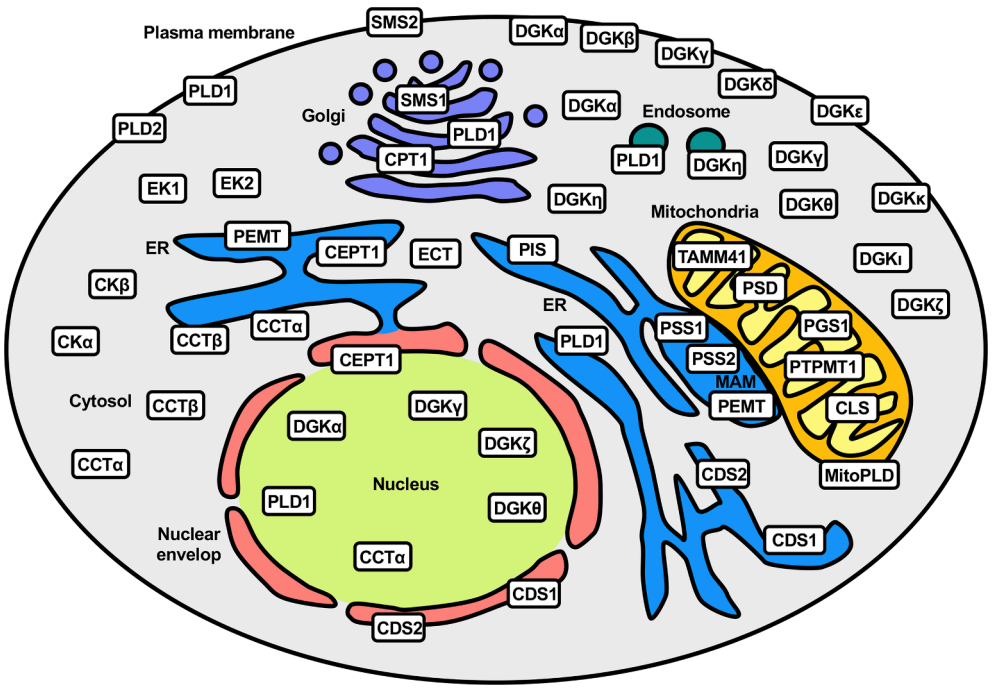

Fig. 4. Intracellular localization of human phospholipid biosynthesis enzymes. CCT, CTP: phosphocholine cytidylyltransferase; CDS, CDP-diacylglycerol synthase; CEPT, choline/ethanolaminephosphotransferase; CK, choline kinase; CLS, cardiolipin synthase; CPT, CDP-choline:diacylglycerol cholinephosphotransferase; DGK, diacylglycerol kinase; ECT, CTP:phosphoethanolamine cytidylyltransferase; EK, ethanolamine kinase; ER, endoplasmic reticulum; MAM, mitochondria-associated membrane; MitoPLD, mitochondria-specific phospholipase D; PEMT, phosphatidylethanolamine *N*-methyltransferase; PGS, phosphatidylglycerophosphate synthase; PIS, phosphatidylinositol synthase; PLD, phospholipase D; PSD, phosphatidylserine decarboxylase; PSS, phosphatidylserine synthase; PTPMT, protein tyrosine phosphatase localized to the mitochondrion; SMS, sphingomyelin synthase; TAMM, mitochondrial translocator assembly and maintenance homolog protein.

Table 1
Enzymes related to PC, PE and PS biosynthesis in human cells.

Gene symbol (Other name)	Chromosome locus	Protein name (Abbreviated name)	Accession No.	Amino acids	Protein type	Intracellular localization
CHKA	11q13.2	Choline kinase alpha (CKα)	NP_001268	457*	Soluble protein	Cytosol
CHKB	22q13.33	Choline kinase beta (CKβ)	NP_005189	395	Soluble protein	Cytosol
PCYT1A (*CTA*, *CCTA*)	3q29	Choline-phosphate cytidylyltransferase alpha (CCTα)	NP_005008	367	Reversible membrane-binding protein	Nucleus, cytosol, and ER
PCYT1B (*CTB*, *CCTB*)	Xp22.11	Choline-phosphate cytidylyltransferase beta (CCTβ)	NP_004836	369*	Reversible membrane-binding protein	Cytosol and ER
CHPT1 (*CPT1*)	12q23.2	Cholinephosphotransferase 1 (CPT1)	NP_064629	406	7-transmembrane protein	Golgi
CEPT1	1p13.3	Choline/ethanolaminephosphotransferase 1 (CEPT1)	NP_006081	416	7-transmembrane protein	ER and nucleus
PEMT	17p11.2	Phosphatidylethanolamine *N*-methyltransferase (PEMT)	NP_680477	236*	3-transmembrane protein	ER (MAM)
ETNK1 (*EKI1*)	12p12.1	Ethanolamine kinase 1 (EK1)	NP_061108	363*	Soluble protein	Cytosol
ETNK2 (*EKI2*)	1q32.1	Ethanolamine kinase 2 (EK2)	NP_060678	386*	Soluble protein	Cytosol
PCYT2 (*ET*)	17q25.3	Ethanolamine-phosphate cytidylyltransferase (ECT)	NP_002852	389*	Soluble protein	Cytosol
SELENOI (*EPT1*)	2p23.3	Ethanolaminephosphotransferase 1 (EPT1)	NP_277040	397	7-transmembrane protein	Unknown
PISD (*PSD*)	22q12.2	Phosphatidylserine decarboxylase (PSD)	NP_055153	375*	Soluble protein	Mitochondria
PTDSS1 (*PSS1*)	8q22.1	Phosphatidylserine synthase 1 (PSS1)	NP_055569	473*	10-transmembrane protein	ER (MAM)
PTDSS2 (*PSS2*)	11p15.5	Phosphatidylserine synthase 2 (PSS2)	NP_110410	487*	7- transmembrane protein	ER (MAM)

* Other isoforms exist.

for approximately 30 % of PC biosynthesis [69]. Transcript variant 1 of the human *PEMT* gene encodes the longer isoform of PEMT (PEMT-L) (236 amino acids), and transcript variants 2 and 3 encode the shorter isoform of PEMT (PEMT-S) (199 amino acids) [68,70,71]. PEMT-L contains a 37-amino-acid extension at the *N*-terminus, which is *N*-glycosylated with high-mannose oligosaccharides and within the ER lumen

[68]. The cellular pool of *S*-adenosylmethionine is mainly in the cytosol, and the *S*-adenosylmethionine-binding site of PEMT is localized on the cytosolic surface of the ER membrane [72]. The enzymatic activity of PEMT-L is much lower than that of PEMT-S [68]. Using HEK293 cells, we have demonstrated that the expression of PEMT-S results in greater increases in PC and ether-linked PC molecular species with longer polyunsaturated chains than that of PEMT-L, whereas PEMT-L expression increases ether-linked PC species with shorter chains [68]. On the basis of these findings, we have suggested a model in which PEMT contains three transmembrane domains, and in which enzyme activity and substrate specificity are regulated by its *N*-terminal region localized in the ER lumen [68]. DeLong et al. have also shown that, in McA-RH7777 rat hepatoma cells expressing rat PEMT (199 amino acids), PC molecules derived from the CDP-choline pathway are mainly composed of medium-chain PC species, whereas PC molecules produced from the PEMT pathway contain more polyunsaturated long-chain PC species than those from the CDP-choline pathway [69].

SM is synthesized by the transfer of phosphocholine from PC to ceramide, which also yields DG [73]. In mammalian cells, most ceramide molecules are converted to SM molecules in the lumen of the *trans*-Golgi by SM synthase 1 (SMS1). Subsequently, the SM molecules are transported to the plasma membrane via vesicular transport. SM generation also occurs at the plasma membranes via SMS2. Conversely, SMase hydrolyzes SM into ceramide and phosphocholine.

3. PE biosynthesis

PE is the second most abundant phospholipid in the mammalian cell membrane. Particularly in the brain, PE constitutes approximately 45 % of total phospholipids [2]. In mammalian cells, PE is produced through the CDP-ethanolamine and PS decarboxylation pathways [2,44]. In the CDP-ethanolamine pathway, PE is synthesized via a sequence of reactions catalyzed by ethanolamine kinase (EK), CTP:phosphoethanolamine cytidylyltransferase (ECT), and CDP-ethanolamine:diacylglycerol ethanolaminephosphotransferase (EPT).

The cytosolic enzyme EK catalyzes the first reaction in the CDP-ethanolamine pathway for PE biosynthesis. Ethanolamine is phosphorylated to phosphoethanolamine by EK. EK1 and EK2 are ethanolamine-specific kinases with negligible choline kinase activity [74,75]. In humans, the EK1 (363 amino acids) and EK2 (386 amino acids) proteins are 67.8 % similar (Table 1). CKα and CKβ phosphorylate choline and ethanolamine [52]. Phosphoethanolamine is also generated by sphingosine-1-phosphate lyase [76].

In the second reaction, CTP:phosphoethanolamine cytidylyltransferase (ECT), a cytosolic enzyme, converts CTP and phosphoethanolamine into CDP-ethanolamine and pyrophosphate [2]. Unlike CCT, human ECT has two catalytic domains [77].

The final reaction of the CDP-ethanolamine pathway is catalyzed by EPT, which combines CDP-ethanolamine with DG to yield PE and CMP. Human CEPT1 has both cholinephosphotransferase and ethanolaminephosphotransferase activities in the ER and nuclear membranes [64]. Human EPT1 has seven predicted transmembrane helices and shows 41.8 % similarity with human CEPT1 at the amino acid level [78]. In contrast with CEPT1, EPT1 is specific for CDP-ethanolamine, but not for CDP-choline [78].

PS decarboxylase (PSD) synthesizes PE from PS in mitochondria [2,44,79]. PSD activity is restricted to the inner mitochondrial membrane [80]. Mammalian PSD undergoes post-translational processing and is then exposed to the outer surface of inner mitochondrial membranes [81–83]. The mature form of PSD is derived from a precursor protein, and the processing at the Leu-Gly-Ser-Thr sequence near its C-terminus is required to form the active enzyme [83]. PS decarboxylation requires the transport of PS synthesized in the ER to the site of PSD in mitochondria, which involves three steps: the movement of PS from the MAM to the outer mitochondrial membrane, the transbilayer movement across the outer mitochondrial membrane, and the transfer from the inner leaflet of the outer mitochondrial membrane to the outer leaflet of the inner mitochondrial membrane [2,44,84]. However, when using the SOSUI ver. 1.11 and TMHMM-2.0 [85,86], both the precursor and mature forms of human PSD were predicted to be soluble and have no transmembrane domain.

In mammals, ethanolamine in the CDP-ethanolamine pathway is derived from dietary sources. In cultured cells supplemented with a medium lacking ethanolamine, PE is largely generated by PS decarboxylation [2,68,87]. In McA-RH7777 and Chinese hamster ovary (CHO)-K1 cells cultured in serum-containing medium, the CDP-ethanolamine pathway is preferred over PS decarboxylation [88]. The CDP-ethanolamine pathway preferentially synthesizes PE species with mono- or di-unsaturated fatty acids at the *sn*-2 position, whereas the PS decarboxylation pathway mainly produces PE species with polyunsaturated fatty acids at the *sn*-2 position [88].

4. PS biosynthesis

PS is a quantitatively minor component of mammalian cell membranes, namely, 2 %-10 % of total phospholipids. In plasma membranes, the majority of PS molecules resides on the inner leaflet of the bilayer [2]. In mammalian cells, PSS catalyzes the exchange of L-serine with the choline moiety of PC or ethanolamine moiety of PE [2,84,89]. Human PSS1 and human PSS2 are 29.0 % identical and 42.6 % similar in their amino acid sequences (Table 1). Human PSS1 is responsible for the base-exchange reactions of both PC and PE, whereas human PSS2 specifically catalyzes the base-exchange reaction of PE [84,90]. The enzymatic activity of PSS2, but not of PSS1, is regulated through feedback inhibition by PS [90,91]. In CHO cells, the residue Arg-97 of PSS2 is important for feedback inhibition [92]. Both murine PSS1 and murine PSS2 are highly enriched in the ER subdomain MAM, which is in contact with the outer mitochondrial membrane [93]. Recently, Miyata and Kuge have provided a 10-transmenbrane topology model of Chinese hamster PSS1, suggesting that PSS1 synthesizes PS in the luminal leaflet of the ER membrane [94]. The amino acid sequence of human PSS1 is 97.3 % identical to that of Chinese hamster PSS1. The programs SOSUI ver. 1.11 and TMHMM-2.0 [85,86] predicted seven transmembrane helices in human PSS2.

5. PA biosynthesis

In mammalian cell membranes, PA is present in small amounts but is a central intermediate in the synthesis of membrane phospholipids and storage acylglycerols [95]. PA is generated in mammalian cells via three different pathways: the de novo pathway involving acyltransferases, the diacylglycerol kinase (DGK) pathway, and the PLD pathway [55,79,95]. PA is converted to DG, another potentially bioactive lipid, by PA phosphatase (PAP) [7,79]. PA is deacylated by phospholipase A_2 to form LPA, which is reconverted to PA by LPA acyltransferase (LPAAT).

In the de novo pathway for PA synthesis, glycerophosphate acyltransferase (GPAT) catalyzes the first step, in which LPA is formed from glycerol-3-phosphate (G3P) and acyl-CoA. GPAT1 and GPAT2 are localized in the outer mitochondrial membrane, and GPAT3 and GPAT4 are localized in the ER membrane [79,96]. Subsequently, using acyl-CoA, LPA is converted into PA, which is catalyzed by the LPAAT activity. LPAAT1, LPAAT2, and LPAAT3 are localized in the ER, whereas LPAAT4 and LPAAT5 are localized in the ER and mitochondria [97].

DGK generates PA via the phosphorylation of DG by using ATP [95,98]. The mammalian DGK family contains ten members classified into five subtypes based on their regulatory domains (Table 2) [98–100]. Each DGK family member contains a catalytic domain in the C-terminal half. In the catalytic domain, the DDGD motif is a putative ATP-binding region [98]. Type I DGK (DGKα, DGKβ, or DGKγ) contains a calcium-binding EF-hand motif and a recoverin homology domain in the *N*-terminus. Type II DGK (DGKδ, DGKη, or DGKκ) is characterized by the separated catalytic domain, four coiled-coil structures, and an N-

Table 2
Enzymes related to PA biosynthesis in human cells.

Gene symbol (Other name)	Chromosome locus	Protein name (Abbreviated name)	Accession No.	Amino acids	Protein type	Intracellular localization
DGKA	12q13.2	Diacylglycerol kinase alpha (DGKα)	NP_001336	735*	Soluble protein	Cytosol, nucleus, and plasma membrane
DGKB	7p21.2	Diacylglycerol kinase beta (DGKβ)	NP_663733	773*	Soluble protein	Plasma membrane
DGKG	3q27.2-q27.3	Diacylglycerol kinase gamma (DGKγ)	NP_001337	791*	Soluble protein	Cytosol, nucleus, and plasma membrane
DGKD	2q37.1	Diacylglycerol kinase delta (DGKδ)	NP_003639	1170*	Soluble protein	Plasma membrane and cytoplasmic vesicle
DGKE	17q22	Diacylglycerol kinase epsilon (DGKε)	NP_003638	567	1-transmembrane protein	Plasma membrane
DGKZ	11p11.2	Diacylglycerol kinase zeta (DGKζ)	NP_003637	929*	Soluble protein	Cytosol and nucleus
DGKH	13q14.11	Diacylglycerol kinase eta (DGKη)	NP_690874	1164*	Soluble protein	Cytosol and endosome
DGKQ	4p16.3	Diacylglycerol kinase theta (DGKθ)	NP_001338	942	Soluble protein	Cytosol and nucleus
DGKI	7q33	Diacylglycerol kinase iota (DGKι)	NP_004708	1065*	Soluble protein	Cytosol
DGKK	Xp11.22	Diacylglycerol kinase kappa (DGKκ)	NP_001013764	1271	Soluble protein	Plasma membrane
PLD1	3q26.31	Phospholipase D1 (PLD1)	NP_002653	1074*	Palmitoylated protein	Golgi, nucleus, ER, plasma membrane, endosomes, and lysosomes
PLD2	17p13.2	Phospholipase D2 (PLD2)	NP_002654	933*	Palmitoylated protein	Plasma membrane
PLD6	17p11.2	Mitochondrial cardiolipin hydrolase (MitoPLD)	NP_849158	252	1-transmembrane protein	Mitochondria

*Other isoforms exist.

terminal pleckstrin homology (PH) domain. The structural characteristic of type III DGK (DGKε) is the presence of a predicted transmembrane helix in the *N*-terminal region. Type IV DGK (DGKζ or DGKι) has four ankyrin repeats near the C-terminus, a myristoylated alanine-rich C-kinase substrate (MARCKS) homology region upstream of the catalytic domain, and a PDZ-binding site at the C-terminus. Type V DGK (DGKθ) contains a proline/glycine-rich domain at the *N*-terminus and a PH domain overlapping with the Ras-associating domain. In their *N*-terminal half, type I, II, III, or IV DGK has two conserved cysteine-rich C1 domains, whereas type V DGK has three C1 domains.

DGK is highly specific for DG as a substrate and does not catalyze ceramide phosphorylation [98]. Human DGKε possesses substrate specificity for DG with an arachidonoyl chain (20:4) at the *sn*-2 position [101]. The activity of pig DGKα is enhanced in the presence of PS, PI, PE, or SM [102]. PS and PI(4,5)P_2 strongly inhibit DGKε activity but increase DGKζ activity [103]. Most types of DGK are localized in the cytosol. DGKα, DGKγ, DGKθ, and DGKζ translocate from the cytosol to the nucleus. Although human DGKα is located in the cytosol and nucleus of resting T cells, interleukin 2 stimulation leads to its translocation to the perinuclear region [104]. Pig DGKα is translocated from the cytosol to the plasma membrane after purinergic receptor stimulation and tocopherol treatment [105,106]. Hepatocyte growth factor-induced plasma membrane translocation of human DGKα requires the tyrosine phosphorylation by Src family kinases [107]. In addition, tyrosine phosphorylation of pig DGKα by c-Abl tyrosine kinase regulates the serum-induced nuclear export of DGKα to the cytosol [108]. Rat DGKβ is mainly localized on the plasma membranes of the neuroblastoma cell line SH-SY5Y [109]. The C1 domain of rat DGKγ is indispensable for its nuclear translocation [110]. By using phorbol ester or ATP as an agonist of purinergic receptors, rat DGKγ is translocated from the cytosol to the plasma membranes in CHO-K1 cells [105]. In HEK293 cells, human DGKδ is translocated from cytoplasmic vesicles to the plasma membrane upon phorbol ester stimulation [111]. Serine phosphorylation within the PH domain by cPKC negatively regulates the plasma membrane translocation of human DGKδ [112]. Human DGKε is localized in plasma membranes [113]. DGKζ is present in the nuclear speckle domains and associated with the nuclear matrix in C2C12 mouse myoblasts [114]. The export of human DGKζ from the nucleus to the cytosol depends on phosphorylation of the MARCKS homology domain by PKCα [115,116]. In response to sorbitol stimulation, human DGKη rapidly translocates from the cytosol to endosomes [117,118]. The translocation of DGKθ to the nucleus is induced by α-thrombin in IIC9 cells, a subclone of CHO cells [119]. Rat DGKι is localized in the cytosol of neurons despite the presence of a nuclear localization signal [120,121]. Human DGKκ persistently resides at the plasma membrane even without stimulation [118,122].

Mammalian PLD catalyzes the hydrolysis of glycerophospholipids, mainly PC, to generate PA. Human PLD1 and human PLD2 share 63.7 % amino acid similarity. Both PLD1 and PLD2 contain two HKD motifs required for the enzymatic catalysis, phox consensus sequence, PH domain, and the PI(4,5)P_2 binding site [7]. PLD1, but not PLD2, has a loop region that functions as a negative regulatory element [123]. PI(4,5)P_2 and PI(3,4,5)P_3, but not other acidic phospholipid classes, activate human PLD1 [124]. The enzymatic activity of human PLD2 is dependent on PI(4,5)P_2 [125]. The activity of human PLD1 is enhanced by PKCα or ADP ribosylation factor-1 and Rho-family GTPases including RhoA, CDC42, and Rac1 [123,124].

PLD1 and PLD2 are palmitoylated on conserved cysteine residues, leading to membrane association [126–128]. PLD1 is distributed throughout the cell, particularly in the Golgi apparatus, nucleus, ER, plasma membrane, endosomes, and lysosomes [7,43,129]. Palmitoylation of rat PLD1 is crucial for epidermal growth factor-induced phosphorylation and activation of caveolin-enriched membranes [130]. PLD2 is primarily localized to the plasma membrane [131,132].

Mitochondria-specific PLD (MitoPLD) hydrolyzes CL to generate PA [8]. The *N*-terminus of MitoPLD penetrates the outer mitochondrial membrane [8]. Unlike PLD1 and PLD2, MitoPLD contains only one HKD motif [8].

6. CDP-DG biosynthesis

CDP-DG is a pivotal intermediate in the production of PI via PI synthase (PIS) in the ER, and PG and CL via phosphatidylglycerophosphate synthase (PGS), phosphatidylglycerophosphate phosphatase (PGPP), and CL synthase (CLS) in the mitochondrial membranes. CDP-DG synthase (CDS) converts PA and CTP into CDP-DG and pyrophosphate. In mammalian cells, CDS1, CDS2, and mitochondrial translocator assembly and maintenance homolog protein 41 (TAMM41) possess CDS activity [55,133,134]. Human CDS1 and human CDS2 are 461- and 445-amino acid membrane proteins, respectively, and share 70.6 % identity and 82.3 % similarity at the amino acid level (Table 3). However, the amino acid sequence of human TAMM41 is 21.3 % or 4.0 % similar to that of human CDS1 or that of human CDS2, respectively. The programs SOSUI ver. 1.11 and TMHMM-2.0 [85,86] predicted that human CDS1 and human CDS2 are 7-transmembrane protein and 8-transmembrane protein, respectively. By contrast, human TAMM41 is predicted to be a soluble protein but not an integral membrane protein.

Human CDS2 exhibits a high preference for 1-stearoyl-2-arachidonoyl PA [135]. By contrast, human CDS1 has no specificity for PA substrates [135]. Both human CDS1 and human CDS2 are inhibited by PI species, especially PI(4,5)P_2 [135]. Human CDS1 and human CDS2 are localized to the ER membrane and nuclear envelope, but not to the mitochondria [3,135,136]. CDS1 is localized to calnexin-enriched microdomains of the ER in A431 human epidermoid carcinoma cells [137]. TAMM41, a peripheral mitochondrial protein, is responsible for mitochondrial CDS activity in H9c2 rat cardiomyoblasts [138].

7. PI biosynthesis

PI is a minor anionic lipid (5 %-10 % of total lipids) in mammalian cells [139]. In the ER, PIS catalyzes PI synthesis from *myo*-inositol and CDP-DG [3,133,139,140]. In human PIS, four or five transmembrane domains were predicted by SOSUI or TMHMM, respectively (Table 3). PIS exhibits no acyl chain specificity toward different CDP-DGs, like CDS1 [141].

Human PIS is localized in the ER tubules and central perinuclear ER-Golgi region, and is particularly enriched in highly mobile ER-derived membrane compartments [136]. Although CDS1 and CDS2 supply CDP-DG for PIS, both CDSs do not exist in highly dynamic compartments containing PIS [136,142]. Furthermore, PI is phosphorylated to form PIPs, including PI(4)P, PI(5)P, PI(3)P, PI(4,5)P_2, PI(3,4)P_2, PI(3,5)P_2 and PI(3,4,5)P_3, which are implicated in various cellular processes including intracellular signaling, protein recruitment, actin cytoskeleton regulation, vesicular trafficking, and cell growth [40,41].

8. PG and CL biosynthesis

In mammalian cells, PG is a minor phospholipid component (<1% of total phospholipids) in many intracellular organelle membranes and located mostly in the mitochondrial and microsomal membranes [79,143]. PG is one of the main components (up to 10 %) of pulmonary surfactants and is predominantly localized in the lamellar body membranes of type II alveolar epithelial cells [17,19]. On the other hand, most CL molecules in mammalian cells are located in the mitochondrial membranes, especially the inner mitochondrial membranes [42]. Notably, cardiac muscle cells contain a high amount of CL (9 %-15 % of total phospholipids) [144]. In the mitochondria of mammalian cells, PG is generated from CDP-DG and G3P via two enzymatic steps, catalyzed by phosphatidylglycerophosphate synthase (PGS) and phosphatidylglycerophosphate phosphatase (PGPP) [39,42,79]. Subsequently, CL synthase (CLS) catalyzes the conversion of PG and CDP-DG into CL [39,42,79]. PG is also a precursor of bis(monoacylglycero)phosphate (BMP), also known as lysobisphosphatidic acid. BMP is a structural isomer of PG and preferentially located in lysosomes and late endosomes [39]. BMP participates in the formation of multivesicular bodies that originated from late endosomes [39].

PGS1 converts CDP-DG to phosphatidylglycerophosphate (PGP) through the exchange of G3P with CMP moiety of CDP-DG [39,42,79,144]. Human PGS1, a 556-amino-acid enzyme, contains the HKD motif, and was predicted by SOSUI and TMHMM to have no transmembrane domain (Table 3). Human PGS1 resides mainly in mitochondria [145].

PGP is rapidly dephosphorylated to produce PG in mitochondria. Human protein tyrosine phosphatase localized to the mitochondrion 1 (PTPMT1) is a PGPP anchored to the matrix side of the inner mitochondrial membrane [42,146,147]. The evolutionarily conserved EEYE loop in PTPMT1 is required for its enzymatic activation [147].

In the mitochondrial inner membrane, CLS catalyzes the condensation of PG and CDP-DG to form CL and CMP [39,42,79]. In rat liver mitochondria, CLS synthesizes CL in the matrix leaflet of the inner mitochondrial membrane [148]. Human CLS was predicted by TMHMM or SOSUI to possess three or seven transmembrane domains, respectively.

9. Regulation of phospholipid biosynthesis with cell growth

Phospholipid biosynthesis is essential for cell cycle progression

Table 3
Enzymes related to PI, PG and CL biosynthesis in human cells.

Gene symbol (Other name)	Chromosome locus	Protein name (Abbreviated name)	Accession No.	Amino acids	Protein type	Intracellular localization
CDS1	4q21.23	CDP-diacylglycerol synthase 1 (CDS1)	NP_001254	461	7-transmembrane protein	ER and nuclear envelop
CDS2	20p12.3	CDP-diacylglycerol synthase 2 (CDS2)	NP_003809	445	8-transmembrane protein	ER and nuclear envelop
TAMM41	3p25.2	Mitochondrial translocator assembly and maintenance homolog protein 41 (TAMM41)	NP_620162	316*	Soluble protein	Mitochondria
CDIPT (*PIS*)	16p11.2	Phosphatidylinositol synthase (PIS)	NP_006310	213*	4- or 5-transmembrane protein	ER
PGS1	17q25.3	Phosphatidylglycerophosphate synthase 1 (PGS1)	NP_077733	556	Soluble protein	Mitochondria
PTPMT1	11p11.2	Phosphatidylglycerophosphatase and protein-tyrosine phosphatase 1 (PTPMT1)	NP_783859	201*	Soluble protein	Mitochondria
CRLS1 (*CLS*)	20p12.3	Cardiolipin synthase (CLS)	NP_061968	301*	3- or 7-transmembrane protein	Mitochondria

*Other isoforms exist.

because the amount of cellular phospholipids doubles prior to cell division. In the murine macrophage cell line BAC1.2F5, Tessner et al. have shown that the mRNA of CCTα and the enzymatic activity of CCT are elevated by colony-stimulating factor-1, which is required for both proliferation and survival of the cells [149]. The mRNA and protein levels of CCTα increase in the rat liver after partial hepatectomy [150]. During the perinatal period in developing rats, the mRNA expression of CCTα and PEMT is positively and negatively associated with liver growth, respectively [151,152]. Jackowski has demonstrated in BAC1.2F5 cells that periodic phospholipid accumulation occurs during the S phase of the cell cycle, whereas CCT activity increases to a maximum in the G_1 phase and gradually declines during the S phase [153]. Golfman et al. have reported that, in C3H10T1/2 mouse embryo fibroblasts, the synthesis of PC increases during the G_1 phase of the cell cycle prior to the enhanced gene transcription of CCTα during the S phase, which can be accounted for by the rapid modulation of PC synthesis by reversible binding of CCTα to cellular membranes without an increase in the amount of CCTα protein [154]. In IIC9 Chinese hamster embryo fibroblasts, CCTα, but not CCTβ, is redistributed from the nucleus to the ER during the G_0 to G_1 transition, which may be required for the rapid synthesis of PC [155]. The promoter of mouse CCTα gene lacks a TATA box but contains a GC-rich region and sites for the binding of transcription factors, including specificity protein-1 (Sp1), Sp3, transcriptional enhancer factor-4, Ets-1, sterol response element-binding protein, and Rb [45,156]. To generate the membrane required for cell division, the phosphorylation of Sp1 ubiquitous transcription factor by cycline-dependent kinase 2 activates CCTα transcription during the S phase of the cell cycle in C3H10T1/2 fibroblasts [157,158]. In COS7 monkey kidney cells, Ets-1 forms a ternary complex with Sp1 in the promoter region to enhance the transcription of CCTα, whereas Net, an Ets-related protein, strongly represses transcription [159]. In NIH3T3 mouse embryonic fibroblasts, the mRNA level of CCTα increases in the S to M phase, accompanied by an increase in the mRNA level of Ets-1 and a decrease in that of Net [159].

During cytokinesis, the cleavage furrow is formed by the interaction of actin filaments with myosin II filaments, and opposing plasma membranes fuse with each other, which is accompanied by the dynamic rearrangement of membrane lipids. Coordinated changes in the arrangement of cytoskeletal proteins and membrane lipids are essential for cell division. Emoto et al. have demonstrated that PE is exposed on the cell surface specifically at the cleavage furrow in dividing CHO-K1 cells, suggesting enhanced scrambling of PE at the cleavage furrow [160]. In CHO-K1 cells, cell surface PE on the cleavage furrow may play crucial roles in contractile ring disassembly and the completion of cytokinesis [161].

The transcription factor X-box-binding protein-1 (XBP-1) is associated with the unfolded protein response triggered by perturbations in ER function. XBP-1(S), generated by the unfolded protein response-mediated splicing of *Xbp-1* mRNA, is necessary and sufficient for the biogenesis of functional ER. XBP-1(S)-induced ER biogenesis in NIH3T3 fibroblasts is associated with increased production of PC and PE and enhanced activities of CCT and CPT, which may be attributed to post-transcriptional and/or post-translational regulation of CCT and CPT proteins [162,163]. XBP-1 is also required for the development of plasma cells, and the expansion of the ER and Golgi compartments is a prerequisite for the high-rate synthesis and secretion of immunoglobulins. In CH12 murine B-lymphoma cells, lipopolysaccharide stimulation leads to increases in IgM secretion, XBP-1(S) expression, and cellular contents of PC and PE [164]. Lipopolysaccharide stimulation of CH12 B-cells upregulates the mRNA expression of CPT1 and increases the amount of CCTα protein owing to the stabilization of protein turnover [164].

In mouse 3T3-L1 cells, the mRNA and protein levels of PEMT increase upon differentiation into adipocytes [165]. The *PEMT* promoter of contains the binding sites for transcription factors, Sp1, Sp3, and Yin Yang 1 [165]. The Sp1 protein level and the interaction of Sp1 with the *PEMT* promoter decrease during the differentiation of 3T3-L1 cells to adipocytes, suggesting that Sp1 is a negative regulator of *PEMT* transcription [165]. In HeLa cells, knockdown of *CDS1* or *CDS2* results in the formation of giant lipid droplets and increases the amount of PA in the lipid droplet fraction, suggesting that CDS1 and CDS2 are key regulators of lipid storage [142].

To further understand the molecular mechanisms of phospholipid metabolism, we have recently developed enzymatic fluorometric assays to measure all major phospholipid classes, namely PC, PE, PS, PA, PI, PG + CL, and SM, using combinations of specific enzymes and 10-acetyl-3,7-dihydroxyphenoxazine (also called Amplex Red) [3,68,145,166–169]. These assays involve several enzymatic steps and fluorometric detection of enzymatically generated hydrogen peroxide using peroxidase and Amplex Red in the final steps (Table 4). These enzymatic fluorometric assays enable the simple, sensitive (picomolar range), and high-throughput quantification of all major phospholipid classes. The assay principles and detailed protocols have been summarized in our previous review [169].

We have used these novel assays to assess the relationship between cellular phospholipid composition and cell growth [4]. Human hepatoblastoma cell line HepG2 has been used to study a wide variety of biological processes [170–174]. The morphology and activity of HepG2 cells are altered by cell growth. To clarify the relationship between cell growth and phospholipid metabolism in HepG2 cells, we have determined the phospholipid class composition of the cells and their intracellular organelles. The ratios of all phospholipid classes, but not that of cholesterol, in HepG2 cells change substantially from the logarithmic phase to the stationary phase of cell growth (Fig. 5). Accompanying cell growth, the cellular PC, PI, and SM ratios increase, whereas the PE, PS, PA, and PG + CL ratios decrease. Notably, the SM ratio increases in the microsomal membranes, the PE ratio increases and the PS ratio decreases in the mitochondrial membranes, and the PA ratio increases and PC ratio decreases in the nuclear membranes depending on cell growth. Additionally, during cell growth, mRNA expression levels of PEMT, PLD1, and PLD2 increase, whereas those of CCTα, CCTβ, CPT1, ECT, PSD, PSS1, DGKκ, CDS1, CLS, and SMS2 decrease. During cell growth, the decrease in the PE ratio may be attributed to the reduced expression

Table 4
Enzymatic fluorometric assays for all major phospholipid classes.

Assay	Using enzyme	Detectable phospholipid class
PC	Glycerophospholipid-specific PLD	PC
	Choline oxidase	Plasmanylcholine
	Peroxidase	
PE	PLD	PE
	Amine oxidase	Plasmenylethanolamine
	Peroxidase	Lysophosphatidylethanolamine
PS	PLD	PS
	L-amino acid oxidase	Lysophosphatidylserine
	Peroxidase	
PA	Lipase	PA
	G3P oxidase	Lysophosphatidic acid
	Peroxidase	
PI	PLD	PI
	myo-Inositol dehydrogenase	Lysophosphatidylinositol
	NADH oxidase	PI(4)P
	Peroxidase	PI(5)P
PG + CL	PLD	PG
	Glycerol kinase	CL
	G3P oxidase	Lysophosphatidylglycerol
	Peroxidase	
SM	SMase	SM
	Alkaline phosphatase	
	Choline oxidase	
	Peroxidase	

CL, cardiolipin; G3P, glycerol-3-phosphate; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PLD, phospholipase D; PS, phosphatidylserine; SM, sphingomyelin; SMase, sphingomyelinase.

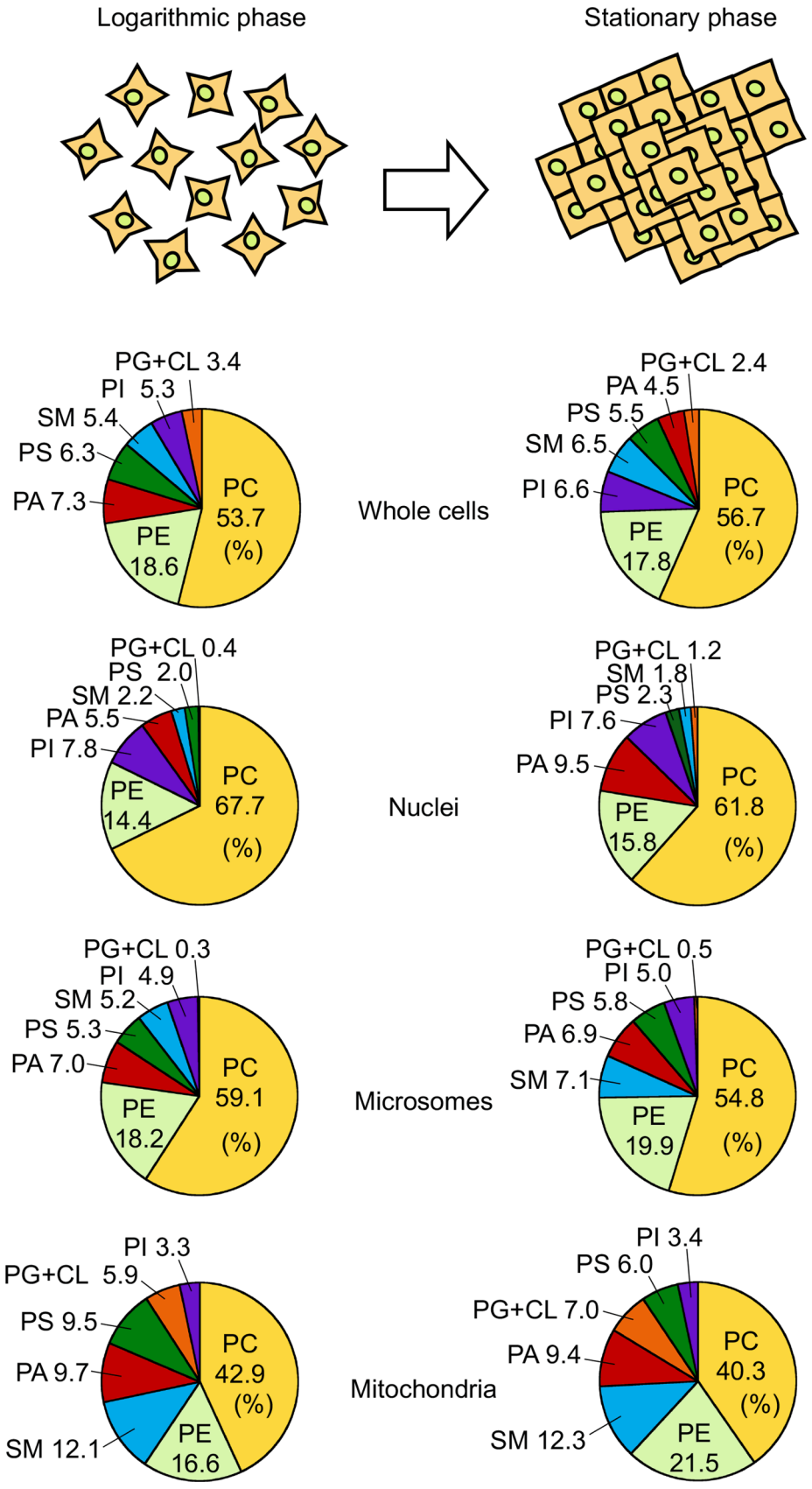

Fig. 5. Changes in phospholipid class compositions of HepG2 cells and their intracellular organelles during cell growth. In the whole cells from the logarithmic phase to the stationary phase of cell growth, the PC, PI, and SM ratios increase, but the PE, PS, PA, and PG + CL ratios decrease. During cell growth, the PA ratio increases and the PC ratio decreases in nuclei, the SM ratio increases in microsomes, and the PE ratio increases and the PS ratio decreases in mitochondria.

of ECT and PSD. The decrease in the PS ratio accompanying cell growth is likely due to PSS1 downregulation. The increase in the PA ratio in the nuclear membranes during cell growth may be ascribed to the upregulation of PLD1 localized to the nucleus. Despite the downregulation of PSD with cell growth, in the mitochondrial membranes, the ratio of PE and that of PS increases and decreases, respectively, suggesting that the transport of PE and PS between the mitochondria and other organelles is regulated by cell growth. These results imply that the phospholipid class composition of intracellular organelle membranes is strictly controlled by cell growth. However, the mechanisms underlying the regulation of enzyme expression and localization during cell growth have not been elucidated.

10. Future directions

Lipid transfer proteins at the membrane contact sites between organelles may play critical roles in the regulation of phospholipid class synthesis in cells. At the ER-plasma membrane contact sites, *N*-terminal domain-interacting receptor 2 (Nir2) contributes to the exchange of PI in the ER and PA in the plasma membrane [175]. Thus, Nir2 may be important for maintaining the PIP pool in the plasma membrane [175]. At the contact sites between the ER and plasma membranes, oxysterol-binding protein-related protein 5 (ORP5) and ORP8, two ER integral membrane proteins, tether the ER to the plasma membrane and work as exchangers mediating the countertransport of PI(4)P from the plasma membrane to the ER and PS from the ER to the plasma membrane [176]. However, mechanisms underlying the transport of phospholipid classes between intracellular organelles remain largely unclear. In addition, several enzymes involved in phospholipid synthesis likely remain unidentified. Three-dimensional structures and catalytic mechanisms of most mammalian enzymes involved in phospholipid biosynthesis have not been elucidated. Moreover, the regulatory mechanisms of gene expression in mammalian cells remain unclear. Further elucidation of phospholipid biosynthetic mechanisms will deepen the insights into physiological processes and help in developing therapies for diseases caused by abnormal phospholipid metabolism.

CRediT authorship contribution statement

Shin-ya Morita: Conceptualization, Funding acquisition, Visualization, Writing – original draft. **Yoshito Ikeda:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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