thematic review

Thematic Review Series: Glycerolipids

Phosphatidylserine and phosphatidylethanolamine in mammalian cells: two metabolically related aminophospholipids

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Abstract Phosphatidylserine (PS) and phosphatidylethanolamine (PE) are two aminophospholipids whose metabolism is interrelated. Both phospholipids are components of mammalian cell membranes and play important roles in biological processes such as apoptosis and cell signaling. PS is synthesized in mammalian cells by base-exchange reactions in which polar head groups of preexisting phospholipids are replaced by serine. PS synthase activity resides primarily on mitochondria-associated membranes and is encoded by two distinct genes. Studies in mice in which each gene has been individually disrupted are beginning to elucidate the importance of these two synthases for biological functions in intact animals. PE is made in mammalian cells by two completely independent major pathways. In one pathway, PS is converted into PE by the mitochondrial enzyme PS decarboxylase. In addition, PE is made via the CDP-ethanolamine pathway, in which the final reaction occurs on the endoplasmic reticulum and nuclear envelope. The relative importance of these two pathways of PE synthesis has been investigated in knockout mice. Elimination of either pathway is embryonically lethal, despite the normal activity of the other pathway. PE can also be generated from a base-exchange reaction and by the acylation of lyso-PE. Cellular levels of PS and PE are tightly regulated by the implementation of multiple compensatory mechanisms.—Vance, J. E. **Phosphatidylserine and phosphatidylethanolamine in mammalian cells: two metabolically related aminophospholipids. *J. Lipid Res.* 2008.** 49: **1377–1387.**

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BIOLOGICAL FUNCTIONS OF PHOSPHATIDYLSERINE AND PHOSPHATIDYLETHANOLAMINE IN MAMMALIAN CELLS

Mammalian cell membranes contain >1,000 different phospholipids. This large mixture of phospholipid species is primarily the result of the distinct fatty acyl chains esterified to the *sn*-1 and *sn*-2 positions of the glycerol backbone as well as the different polar head groups attached to the *sn*-3 position of the glycerol backbone. The amounts of the various phospholipids in a membrane define the fluidity of the membrane and, consequently, the functions of the embedded proteins. Phosphatidylcholine is the most abundant phospholipid in mammalian cell membranes, constituting 40–50% of total phospholipids. The second most abundant mammalian membrane phospholipid is phosphatidylethanolamine (PE), which constitutes 20–50% of total phospholipids. In the brain, ~45% of total phospholipids are PE, whereas in the liver, only ~20% of total phospholipids are PE. Phosphatidylserine (PS) is a quantitatively minor membrane phospholipid that makes up 2–10% of total phospholipids. The metabolic interrelationships among PS, PE, and phosphatidylcholine are depicted in **Fig. 1**. Additional relatively minor mammalian membrane phospholipids include phosphatidylinositol, sphingomyelin, and the mitochondria-specific phospholipid, cardiolipin.

Different types of mammalian cells and tissues have characteristic phospholipid compositions. For example, the brain is enriched in the two aminophospholipids PE and PS compared with other tissues. In the brain, and particularly in the retina (1), the acyl chains of PS are highly enriched in docosahexaenoic acid (22:6n-3) (2–4). In human gray matter, 22:6n-3 accounts for >36% of the fatty acyl species of PS (5–7). Because 22:6n-3 appears to be essential for the normal development and functioning of the nervous system (6, 8–11), it is likely that PS plays

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Abbreviations: CHO, Chinese hamster ovary; ER, endoplasmic reticulum; MAM, mitochondria-associated membrane; PE phosphatidylethanolamine; PS, phosphatidylserine; PSD, phosphatidylserine decarboxylase.

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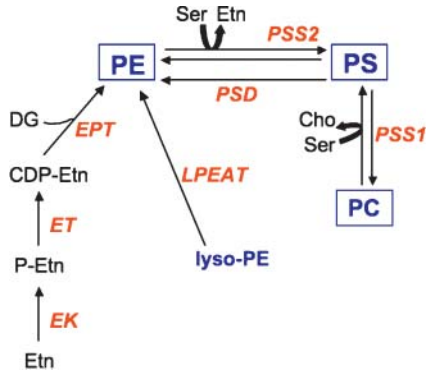

Fig. 1. The metabolism of phosphatidylserine (PS) and phosphatidylethanolamine (PE) in mammalian cells. PS is made in elements of the endoplasmic reticulum (ER) by two base-exchange enzymes, PS synthase-1 (PSS1) and PS synthase-2 (PSS2), that exchange the choline (Cho) and ethanolamine (Etn) head groups of phosphatidylcholine (PC) and PE, respectively, for serine (Ser). In the CDP-ethanolamine pathway for PE synthesis, ethanolamine is phosphorylated by ethanolamine kinase (EK) to produce phosphoethanolamine, which is subsequently converted to CDP-ethanolamine by the action of CTP:phosphoethanolamine cytidylyltransferase (ET). In the final step of this pathway, CDP-ethanolamine combines with 1,2-diacylglycerol in a reaction catalyzed by CDP-ethanolamine:1,2-diacylglycerol ethanolaminephosphotransferase (EPT), an enzyme of the ER and nuclear envelope. Another major PE biosynthetic pathway occurs only in mitochondria and uses phosphatidylserine decarboxylase (PSD), which decarboxylates PS to PE. PE can also be produced from lyso-PE, in a reaction most likely catalyzed by an acyl-CoA-dependent acyltransferase (LPEAT).

an important role in the nervous system and in vision (reviewed in Ref. 4).

The different organelles within mammalian cells also have distinct phospholipid compositions. In mitochondria, particularly in the inner membrane, the PE content is significantly higher than in other organelles. Not only do different organelle membranes have different phospholipid contents, but the two leaflets of the membrane bilayer also have distinct phospholipid compositions. For example, in the plasma membrane, PS and PE are asymmetrically distributed across the bilayer such that the great majority (>80%) of these aminophospholipids are normally confined to the inner leaflet, whereas phosphatidylcholine and sphingomyelin are enriched on the outer leaflet.

Phospholipids were, for many years, thought to play primarily structural roles in biological membranes. A large number of recent studies have revealed, however, that these lipids mediate important regulatory functions in cells, partly because of their ability to be converted into key lipid second messengers such as diacylglycerol, inositol-1,4,5-trisphosphate (12, 13), lyso-phosphatidic acid, and arachidonic acid. PS and PE are metabolically related, as depicted in Fig. 1.

PS is an important precursor of mitochondrial PE, which is produced by the mitochondrial enzyme phosphatidylserine decarboxylase (PSD) (see below) (14). As noted above, in the plasma membrane of mammalian cells PS normally resides almost entirely on the inner leaflet of the bilayer. In the past decade, PS has become a major focus of interest because during the early phases of apoptosis PS becomes externalized on the outside of cells. The surface exposure of PS is believed to be one of the recognition signals by which apoptotic cells are removed by phagocytes (15–17), although the identity of a PS receptor on macrophages remains controversial (18–23). The asymmetric transbilayer distribution of PS in the plasma membrane of mammalian cells is thought to be established and maintained by a continuous unidirectional transbilayer movement of PS from the external surface to the cytosolic surface of the plasma membrane. This process requires ATP, but the aminophospholipid translocase that mediates the "flipping" of PS has not been unambiguously identified. The properties of this protein indicate that it is a member of the P-type ATPase family of transporters that is activated by PS (24). Bidirectional phospholipid transporters called scramblases are also located on the plasma membrane, and these proteins can randomize the distribution of PS and other lipids across the bilayer. The scramblases are activated by calcium and do not require ATP for activity (25). Thus, the exposure of PS on the outside of cells undergoing apoptosis is likely to be promoted by reduced activity of the aminophospholipid translocase combined with increased scramblase activity. The induction of PS exposure on cell surfaces is not restricted to apoptotic cells. For example, the exposure of PS on the surface of activated platelets initiates the blood-clotting cascade (17, 26–28), as the proteolytic activity of the factor VIIa-tissue factor complex requires very high local concentrations of PS (29). Moreover, PS becomes exposed on the outside of sperm during their maturation (30, 31).

Another function of PS is as a cofactor that activates several key signaling proteins, including protein kinase C (32, 33), neutral sphingomyelinase (34), and cRaf1 protein kinase (35), as well as Na^+/K^+ ATPase (35) and dynamin-1 (36). Intriguingly, a highly specific interaction of PS with some Hsp70 heat shock proteins induces the formation of ion channels in the plasma membrane (37). Furthermore, a recent report has shown that PS can direct proteins that are moderately positively charged to membranes of the endocytic pathway (38).

PE also performs numerous biological roles beyond serving a structural role in membranes. For example, PE metabolism in the heart appears to be important, because the asymmetrical transbilayer distribution of PE in sarcolemmal membranes is altered during ischemia, leading to sarcolemmal disruption (39). PE might also play a role in hepatic lipoprotein secretion, because nascent, intracellular very low density lipoproteins that move through the secretory pathway are highly enriched in PE compared with the lipoproteins that are secreted from hepatocytes (40, 41). In addition, PE is required for contractile ring disassembly at the cleavage furrow of mammalian cells during cytokinesis (42). In the yeast *Saccharomyces cerevisiae*, it has been demonstrated that the delivery of cytoplasmic

proteins to the vacuole depends on PE and that the starvation-inducible autophagy protein, Atg8p, binds covalently to PE (43). A role for PE in membrane fusion and fission events has been recognized for many years (44, 45). Some of the biological properties of PE, such as its role in membrane fusion/fission, might be related to the ability of PE to form hexagonal II phases in membranes (reviewed in Ref. 46). PE is also the donor of the ethanolamine moiety of the glycosylphosphatidylinositol anchors of many cell surface signaling proteins (47) and is a precursor of anandamide [*N*-arachidonoylethanolamine (48)], which is a ligand for cannabinoid receptors in the brain (49).

PS SYNTHESIS

In mammalian cells, PS is synthesized by a calcium-dependent reaction (50) in which the polar head group of an existing phospholipid (i.e., the choline moiety of phosphatidylcholine or the ethanolamine moiety of PE) is replaced by L-serine (Fig. 1). In prokaryotes and yeast, PS is made by a completely different pathway in which CDP-diacylglycerol reacts with serine (51–53). The CDP-diacylglycerol pathway for PS synthesis has not been detected in mammalian cells, although, interestingly, plants use both the base-exchange pathway and the CDP-diacylglycerol pathway to make PS (54).

Two mammalian PS synthases

Mammalian cells express two distinct serine-exchange activities. The first indication that two mammalian PS synthase genes existed was the partial purification of a rat brain enzymatic activity that synthesized PS by a serine-exchange reaction with PE but not with phosphatidylcholine (55). An epitope-tagged version of this protein has now been purified to near homogeneity (56). The existence of two mammalian *Pss* genes was confirmed when mutant Chinese hamster ovary (CHO) cells, which had the capacity to synthesize PS from PE but not from phosphatidylcholine, were generated in the laboratories of Nishijima (PSA-3 cells) (57) and Voelker (M9.1.1 cells) (58). The defective choline-exchange activity was called PS synthase-1, and the residual serine-exchange activity, which uses PE as substrate, was named PS synthase-2. Radiolabeling experiments and in vitro enzyme assays in PS synthase-1-deficient CHO cells revealed that the rate of PS synthesis was 35–55% lower than that in parental cells, and the mass of both PS and PE was reduced correspondingly (57, 58). The growth of cells that lacked PS synthase-1 was severely impaired in the absence of supplementation with PS, PE, or ethanolamine. Thus, mammalian cells express PS synthase-1 activity that synthesizes PS from PC. The second serine-exchange activity, PS synthase-2, is encoded by a distinct gene and catalyzes the exchange of serine with PE to make PS (Fig. 1). cDNAs encoding PS synthase-1 and PS synthase-2 from hamster (59, 60) and mouse liver (61, 62) were cloned. The murine and human PS synthase-1 genes reside on chromosomes 13 and 8, respectively. When PS synthase-2 activity was overexpressed in CHO cells, the ethanolamine-exchange activity was increased by 10-fold, whereas the choline-exchange activity remained unchanged (60), supporting the view that PS synthase-2 catalyzes ethanolamine but not choline exchange. Additional evidence that the putative PS synthase-1 cDNA encodes PS synthase-1 was obtained when choline-exchange activity was eliminated by the immunoprecipitation of cell lysates with an antibody raised against a C-terminal peptide corresponding to the predicted PS synthase-1 sequence (63). In other experiments, overexpression of PS synthase-2 in PS synthase-1-deficient cells eliminated the requirement for exogenously added PS (62), indicating that PS synthase-2 can substitute for PS synthase-1 in CHO cells. Cells lacking PS synthase-1 activity (i.e., PSA-3 cells) were further mutagenized, resulting in the production of a mutant cell line in which PS synthase-2 mRNA was reduced by 80%. Consequently, the total PS synthase activity of these cells was ~95% lower than that in parental cells. These doubly mutated cells were viable only when supplied with an exogenous source of PS (60).

A fundamental question arising from the discovery of two PS synthase genes is: why do mammalian cells possess two different PS synthases? Do these synthases perform distinct functions in cells or does the duplication confer merely a backup mechanism? Many examples are known in mammalian cells in which either the same or a similar enzymatic reaction for the synthesis of a specific phospholipid is encoded by distinct genes, or more than one biosynthetic pathway exists for a single phospholipid (64). The tissue distribution of the mRNAs encoding the two PS synthases is different, suggesting that each PS synthase might have a specific function. Whereas PS synthase-1 is expressed ubiquitously throughout mouse tissues and is particularly abundant in the kidney, liver, and brain, the mRNA encoding PS synthase-2 is most highly expressed in the testis, with much lower levels of expression in other tissues such as brain and heart (62, 65, 66). In addition, there are several indications that the two PS synthases differentially regulate phospholipid metabolism. For example, overexpression of PS synthase-1 activity in hepatoma cells increased the rate of incorporation of [^{3}H]serine into PS, consistent with the idea that the amount of PS synthase-1 is rate-limiting for PS synthesis (61). Nevertheless, the cellular content of PS and PE was not increased. The cells were apparently able to compensate for the increased rate of PS synthesis by enhancing the conversion of PS to PE via PSD and by reducing the formation of PE from the CDP-ethanolamine pathway (Fig. 1) (61). In contrast, an equivalent level of overexpression of PS synthase-2 activity in hepatoma cells did not increase the rate of incorporation of [^{3}H]serine into PS or the conversion of PS to PE, nor did it decrease the rate of synthesis of PE from CDP-ethanolamine (62). One potential explanation for the different responses of PS synthesis to the increased expression of PS synthase-1 and -2 is that the amount of PS synthase-1 is rate-limiting for PS synthesis,

whereas PS synthase-2 is normally present in excess of its requirement for maintaining normal PS levels. Consequently, increased expression of PS synthase-2 would not be expected to increase the rate of PS synthesis. These studies clearly demonstrate that mammalian cells have the ability to implement compensatory mechanisms to ensure that constant, optimum levels of PE and PS are maintained.

PS and apoptosis

PS exposure on the cell surface is an early event in apoptosis. The PS that is externalized during apoptosis originates from a pool of newly synthesized PS (67, 68). The rate of PS synthesis has been reported to be increased by ~2-fold during apoptosis (68, 69), although there is no direct evidence that PS externalization is required for the initiation of apoptosis. However, overexpression of either PS synthase-1 or PS synthase-2 in CHO cells reduced the number of cells undergoing apoptosis in response to ultraviolet irradiation (70), suggesting that the synthesis of PS might protect against apoptosis. When Neuro2a (neuron-like) cells were incubated with docosahexaenoic acid (22:6n-3), the PS content of the cells increased and the number of apoptotic cells was reduced concomitantly (71). The mechanism by which survival was promoted is that an increased PS content of the cells increased the translocation of Raf-1 (a kinase that is involved in apoptosis) (72, 73) to membranes. In other experiments, the requirement of PS synthase-1 and PS synthase-2 to provide the pool of PS that is externalized during apoptosis was investigated in CHO cell mutants that lacked either PS synthase-1 (58, 74) or PS synthase-2 (75) or that were deficient in both PS synthases (i.e., contained only 5% of normal serine-exchange activity) (75). In all of these cell lines, the externalization of PS occurred normally upon the induction of apoptosis with staurosporine (76), implying that only very low rates of PS synthesis are required for the progression of apoptosis and the accompanying exposure of PS on the cell surface. In addition, the studies demonstrated that the PS that is externalized is not derived specifically from either isoform of PS synthase.

Other properties of PS synthases

The predicted amino acid sequences of the two PS synthases are ~30% identical (59–62) but, not surprisingly, in light of the distinct reactions catalyzed, are completely different from those of the PS synthases of bacteria and yeast. Little is known about the amino acid residues required for PS synthase activity. However, the individual replacement of 66 polar amino acids of PS synthase-1 with alanines revealed that several amino acids distributed throughout the protein are required for maximum protein stability/activity. Moreover, these studies showed that the binding of L-serine to the enzyme requires asparagine-209 (77). Both PS synthase proteins are predicted to contain multiple membrane-spanning domains (61, 75), consistent with the finding that serine-exchange activity is present on microsomal membranes (63, 78–81). Surprisingly, however, both PS synthase proteins are largely absent from the bulk of the endoplasmic reticulum (ER) but are restricted to a domain of the ER called "mitochondria-associated membranes" (MAMs) (82). MAMs constitute a subdomain of the ER that comes into transient contact with mitochondrial outer membranes (81) and mediates the import of PS into mitochondria (**Fig. 2**) (81–85). PS synthase activity is enriched by ~4-fold in MAMs compared with the bulk of the ER (81, 82, 86). MAMs have been isolated from several types of mammalian cells (81, 86–89) as well as yeast (90, 91). A specific marker protein for MAMs in primary hepatocytes is phosphatidylethanolamine *N*-methyltransferase-2 (82, 92). Increasing evidence indicates that the juxtaposition of MAMs with mitochondria also regulates the exchange of calcium between these two organelles (93–97).

Little is known about the mechanisms that regulate PS synthesis in mammalian cells. Surprisingly, no information is available on the transcriptional regulation of expression of either PS synthase gene (*Pss*). Some early experiments suggest that in rat brain, PS synthesis is regulated by protein kinase C-mediated phosphorylation (98). An elegant feedback mechanism for regulating PS synthesis was described in CHO cells, in which an increased cellular content of PS reduced the rate of PS synthesis, as measured by the incorporation of $^{32}P_i$ into PS (99). A mutant CHO cell line was subsequently isolated in which the rate of PS synthesis, and the amount of PS, were 2.5-fold higher than in parental CHO cells, and the rate of PS synthesis was not attenuated by PS (100). These data suggest that the capacity for end product inhibition of PS synthesis had been eliminated in the mutant cells. Point mutations were subsequently identified in PS synthase-1 (arginine-95) (56,

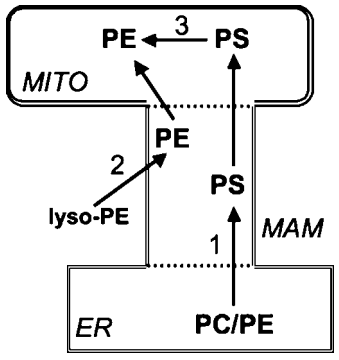

Fig. 2. A model for the import of PS into mitochondria for decarboxylation to PE in mammalian cells. PS is synthesized primarily in mitochondria-associated membranes (MAMs), a specialized domain of the ER, via two base-exchange enzymes (1), PS synthase-1 and PS synthase-2. These synthases use phosphatidylcholine (PC) and PE, respectively, as substrates. Newly made PS is imported into mitochondria (MITO) to the site of PSD on the outer aspect of mitochondrial inner membranes (3) via a transient interaction between MAMs and mitochondria. PE is also likely to be made in MAMs from lyso-PE via a putative acyl-CoA-dependent acyltransferase (2), and the resulting PE is subsequently imported into mitochondria.

101) and PS synthase-2 (arginine-97) (102) that conferred resistance to the feedback inhibition of PS synthesis. It is likely that PS inhibits the serine-exchange activity of PS synthase-1 and PS synthase-2 by acting directly on the protein (56).

PS synthase knockout mice

A powerful tool for understanding the function of specific genes in whole mammals is the generation of knockout mice. To examine the physiological requirement for PS synthase-2, *Pss2*$^{-/-}$ mice were produced. These mice appeared to be outwardly normal (66), demonstrating that PS synthase-2 is not essential for mouse development or viability. Female *Pss2*$^{-/-}$ mice were fertile, but in *Pss2*$^{-/-}$ males testis size was smaller than in *Pss2*$^{+/+}$ littermates. Approximately 10% of the *Pss2*$^{-/-}$ male mice were infertile, with atrophied testes and spermatic ducts lacking spermatocytes. Consistent with a defect in the function of Sertoli cells, the main type of cells in the testis that express PS synthase-2, the level of follicle-stimulating hormone in the plasma of male *Pss2*$^{-/-}$ mice was higher than in *Pss2*$^{+/+}$ mice. Although cases of male infertility in humans have not been attributed to defects in the PS synthase-2 gene, it is possible that mutations in this gene might be responsible for some male subfertility. Despite a marked reduction in total PS synthase activity in all PS synthase-2-deficient mouse tissues, the amounts of PS and PE were normal. Hepatocytes from *Pss2*$^{-/-}$ mice are apparently able to maintain PS levels by increasing the activity, but not the mRNA, of PS synthase-1 and by concomitantly attenuating the rate of PS degradation (103). Viable mice lacking PS synthase-1 have also now been generated and exhibit no obvious phenotype; male and female *Pss1*$^{-/-}$ mice are fertile (D. Arikketh and J. E. Vance, unpublished data).

PE SYNTHESIS

Mammalian cells use two major pathways for PE biosynthesis: the CDP-ethanolamine pathway and the PS decarboxylation pathway (Figs. 1, 2). PE can also be made by a base-exchange reaction catalyzed by PS synthase-2, although this source of PE is generally considered to be quantitatively insignificant (104). In addition, lyso-PE can be acylated to PE by a lyso-PE acyltransferase activity (Figs. 1, 2) (105). In yeast, this acyltransferase activity is highly enriched in MAMs and has been attributed to the acyl-CoA-dependent acyltransferase Ale1p (106–108). Although the mammalian ortholog of Ale1 has not yet been identified, a family of uncharacterized related genes is present in mammals (107, 108). The relative contribution of the PE biosynthetic pathways to cellular PE content has not been firmly established, but it appears to depend on the cell type. In rat liver/hepatocytes and hamster heart, the CDP-ethanolamine pathway has been reported to produce the majority of PE (109–112). In contrast, in many types of cultured cells, >80% of PE is apparently made from the decarboxylation of PS via PSD, even when ethanolamine is provided in the culture medium as a substrate for the CDP-ethanolamine pathway (74, 113, 114). It should be noted, however, that in all studies in which the relative contribution of these two pathways has been evaluated the pool of the immediate precursor of PE was assumed to be homogenously labeled from a radioactive precursor; this assumption is not necessarily valid (115). All molecular species of PE can be made from both pathways in hepatoma cells and CHO cells, although the CDP-ethanolamine pathway preferentially synthesizes PE containing monounsaturated or diunsaturated acyl chains at the *sn*-2 position, whereas the PSD pathway preferentially makes PE containing polyunsaturated acyl chains at the *sn*-2 position (112).

The CDP-ethanolamine pathway

Ethanolamine is a required precursor of PE synthesis via this pathway. Ethanolamine is required for the growth and survival of some cell types, such as hepatocytes (116), keratinocytes (117), and mammary carcinoma cells (118), although this requirement is not necessarily related to the biosynthesis of PE via the CDP-ethanolamine pathway. Plants, but not mammalian cells, produce ethanolamine via a direct decarboxylation of serine (119). Consequently, ethanolamine that is used for PE synthesis in animals must be provided from dietary sources. In addition, small amounts of ethanolamine are produced from the degradation of sphingolipids via the action of sphingosine phosphate lyase (120, 121). The CDP-ethanolamine pathway for PE synthesis was elucidated by Kennedy and Weiss in 1956 (122) and parallels the CDP-choline pathway for phosphatidylcholine synthesis. Mammalian genes encoding the three enzymes of the CDP-ethanolamine pathway have been identified (reviewed in Ref. 123).

The first reaction of the CDP-ethanolamine pathway is catalyzed by the cytosolic enzyme ethanolamine kinase (Fig. 1) (124). One isoform of ethanolamine kinase phosphorylates both ethanolamine and choline. In addition, a cDNA encoding a human ethanolamine kinase that lacks significant choline kinase activity has also been cloned (124). Mice lacking the ethanolamine-specific kinase have been generated (125). In these mice, the PE content of the liver was not decreased but litter size was reduced and ~20% of the pups died perinatally. Thus, it appears that the dual specificity ethanolamine/choline kinase is able, at least partially, to substitute for the ethanolamine-specific kinase in these knockout mice.

The rate-limiting reaction of the CDP-ethanolamine pathway for PE synthesis is catalyzed by another cytosolic enzyme, CTP:phosphoethanolamine cytidylyltransferase (the product of the *Pcyt2* gene in mice), which converts phosphoethanolamine into CDP-ethanolamine (Fig. 1) (126–129). Under some metabolic conditions, however, the reaction catalyzed by ethanolamine kinase has been reported to be rate-limiting for PE synthesis (124). The *Pcyt2* mRNA is most highly expressed in liver, heart, and skeletal muscle. In contrast to CTP:phosphocholine cytidylyltransferase, which is encoded by two genes and exists

in four isoforms in the mouse (130, 131), the cytidylyltransferase of the CDP-ethanolamine pathway is encoded by only a single gene that has extensive regions of homology to the corresponding enzyme of the CDP-choline pathway. One difference between the cytidylyltransferases that participate in phosphatidylcholine and PE synthesis is that CTP:phosphoethanolamine cytidylyltransferase contains two copies of the putative catalytic domain, whereas CTP:phosphocholine cytidylyltransferase contains only a single copy of this motif (128). Moreover, unlike CTP:phosphocholine cytidylyltransferase, the activity of CTP:phosphoethanolamine cytidylyltransferase does not reside in the nucleus and is not regulated by reversible translocation between a soluble form and a membrane-associated form (reviewed in Ref. 132). Mice lacking CTP:phosphoethanolamine cytidylyltransferase do not survive during development, although *Pcyt2*$^{+/-}$ mice appear outwardly normal (133). Thus, although *Pcyt2*$^{-/-}$ mice likely express normal levels of PSD, the PS decarboxylation pathway for PE synthesis cannot substitute for a complete deficiency of the CDP-ethanolamine pathway.

The final step of the CDP-ethanolamine pathway, in which CDP-ethanolamine reacts with diacylglycerol, is catalyzed by an integral membrane protein of the ER and nuclear envelope, CDP-ethanolamine:diacylglycerol ethanolaminephosphotransferase (134, 135) (Fig. 1). A human cDNA encoding both choline- and ethanolaminephosphotransferase activity has been isolated (134, 135). The corresponding mRNA is ubiquitously expressed in all human tissues examined, and the enzymatic activity resides primarily in the ER (80, 136). A related human cDNA with 60% sequence identity to the dual specificity choline/ethanolamine phosphotransferase was also cloned, but this cDNA appears to encode a protein that exhibits only cholinephosphotransferase activity (135). Thus, until recently, it was widely assumed that the dual specificity choline/ethanolamine phosphotransferase provided all of the mammalian ethanolaminephosphotransferase activity. However, this conclusion is probably not valid, because another cDNA that encodes a human CDP-ethanolamine-specific phosphotransferase activity was isolated recently (137). The mRNA encoding this protein is widely expressed in human tissues, including the cerebellum in the brain. Expression of the corresponding cDNA in *Escherichia coli* demonstrated that the gene product uses CDP-ethanolamine to produce PE.

The PS decarboxylation pathway

In contrast to PE synthesis from CDP-ethanolamine, the final step of which occurs on ER membranes, the production of PE via the decarboxylation pathway is restricted to mitochondria. Thus, at least two spatially separated pools of PE might exist: one made in the ER, the other made in mitochondria. The mammalian PSD protein is located on the external leaflet of mitochondrial inner membranes (138, 139). PSD is a member of a small family of decarboxylases that contain an unusual pyruvoyl prosthetic group (140). The catalytically active form of PSD is produced by an autocatalytic proteolysis reaction in which a precursor protein is cleaved between a glycine and a serine residue within a LGST motif to generate two subunits (141); the serine is converted into a pyruvoyl moiety at the N terminus of the resulting α subunit. Complementation experiments in a mutant CHO cell line lacking PSD activity identified a cDNA encoding PSD (142). In contrast to mammalian cells, in which all PSD activity appears to originate from a single gene, two PSD genes are expressed in yeast. Thus, yeast contains two distinct PSD proteins with no sequence similarity, each exhibiting PSD activity (143, 144). One of the yeast isoforms, Psd1p, is restricted to the mitochondria, like the mammalian PSD, whereas the other isoform, Psd2, is located in the Golgi/vacuole.

The PS that is used as the substrate for PSD is produced in the ER and MAMs (Fig. 2). The rate-limiting step in the conversion of PS to PE is the transport of newly synthesized PS to the site of PSD in mitochondria (145). This transfer requires, first, the movement of newly made PS to the mitochondrial outer membrane, followed by transbilayer movement across the mitochondrial outer membrane, and, finally, transfer to the active site of PSD on the outer leaflet of mitochondrial inner membranes. Although the mechanisms of these translocations of PS have not been defined unequivocally, one likely possibility, for which there is some evidence, is that the transfer of PS from MAMs to mitochondrial outer membranes occurs via transient membrane contact sites (Fig. 2) (81, 86, 146). The transfer of PS between outer and inner mitochondrial membranes might also occur via membrane contact sites (87, 147). A mutant CHO cell line has been isolated that has a defect in one of the steps involved in the transfer of PS to the site of PSD (148), but to date the gene involved has not been identified. In yeast, a ubiquitin ligase, Met30p (91), is required for the import of newly synthesized PS into mitochondria for decarboxylation (reviewed in Ref. 149). The precise role of Met30p in this process is not entirely clear, but the ubiquitination of proteins is known to regulate multiple membrane trafficking events in addition to its role in protein degradation (150).

Compared with other organelle membranes, the mitochondrial inner membrane is enriched in PE. Almost all PE in mitochondria appears to be made in situ in mitochondria, whereas very little PE is imported from the ER (86). PSD-deficient mice do not survive beyond embryonic day 9, indicating that production of PE from the PSD pathway is essential for mouse development (151). In *Psd*$^{-/-}$ embryonic fibroblasts, the mitochondria are fragmented and aberrantly shaped, consistent with a defect in mitochondrial fusion. It is likely that elimination of PSD reduces the mitochondrial content of PE, resulting in mitochondrial abnormalities and embryonic lethality. In contrast, *Psd*$^{+/-}$ mice appear normal (151); their mitochondria exhibit normal morphology, and the PE content of the liver and other tissues of *Psd*$^{+/-}$ mice is normal. The amount and activity of the CTP:phosphoethanolamine cytidylyltransferase are increased in these mice, presumably in an attempt to compensate for the lack of PSD. Even when additional ethanolamine is provided in the diet of

pregnant female mice, the CDP-ethanolamine pathway cannot substitute for PSD during mouse development. It is possible that supplementation of $Psd^{-/-}$ cells with lyso-PE rather than ethanolamine would restore normal mitochondrial function, because lyso-PE is far more effective than ethanolamine in preventing mitochondrial defects in PSD-deficient yeast (106). In the yeast *S. cerevisiae*, PE is an essential phospholipid (152). PE made at nonmitochondrial sites via the CDP-ethanolamine pathway or by Psd2 in the Golgi/vacuole does not fully satisfy the mitochondrial requirement for PE made by the mitochondrial PSD, Psd1 (106). The observations in $Psd^{-/-}$ mice and $Pcyt2^{-/-}$ mice indicate that the CDP-ethanolamine and PSD pathways for PE biosynthesis are each essential for mouse development. Alternatively, it is possible that both pathways together are required to maintain a threshold level of PE.

In certain cell types (e.g., inflammatory cells, neurons, and tumor cells), up to 70% of the "PE" pool contains an ether linkage, rather than an acyl linkage, at the *sn*-1 position. The ether lipids that contain a vinyl group (a 1′ *cis* double bond) are called plasmalogens. The biosynthesis of ethanolamine plasmalogen occurs by an unusual series of reactions, some of which occur on peroxisomes and others on the ER (reviewed in Ref. 153). This biosynthetic pathway includes a unique reaction in which an acyl group at the *sn*-1 position of acyl-dihydroxyacetone phosphate is replaced with an alkyl group. Whether or not plasmalogen synthesis uses precursors from both the CDP-ethanolamine pathway and the PSD pathway is unclear. When radiolabeled serine or ethanolamine was injected into rats, the CDP-ethanolamine pathway was used by heart, liver, and kidney for the synthesis of both PE and ethanolamine plasmalogen, whereas the PSD pathway was used solely for PE, but not plasmalogen, synthesis (154). In contrast, serine served as a precursor of the ethanolamine head group of both PE and ethanolamine plasmalogen in cultured C6 glioma cells (155).

The regulation of PSD activity, either at the level of gene expression or by posttranslational mechanisms, has not been reported.

CONCLUSION

New information on PS and PE has elevated these two phospholipids from obscurity to prominence in biology and revealed that these two aminophospholipids play key roles in many biochemical and physiological processes in mammalian cells. The generation of mouse models in which the genes of the PS and PE biosynthetic pathways have been disrupted has significantly contributed to our understanding of the metabolic interrelationships between these two phospholipids. Knockout mice in which genes of the CDP-ethanolamine and PSD pathways have been individually disrupted have provided support for the idea that pools of phospholipids can be compartmentalized on the basis of their biosynthetic origin. Moreover, several recent studies have established that the homeostasis of PS and PE in mammalian cells is rigorously maintained by the implementation of compensatory mechanisms.

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