Phosphatidylserine and Phosphatidylethanolamine in Mammalian Cells: Two Metabolically-related Aminophospholipids

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Key words

apoptosis, phosphatidylserine decarboxylation, phosphatidylserine synthase, CDP-ethanolamine pathway

Abbreviations

CHO, Chinese hamster ovary; ER, endoplasmic reticulum; MAM, mitochondria-associated membranes; PS, phosphatidylserine; PSD, phosphatidylserine decarboxylase; PE phosphatidylethanolamine

Footnotes

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Abstract

Phosphatidylserine (PS) and phosphatidylethanolamine (PE) are two aminophospholipids whose metabolism is inter-related. 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 pre-existing 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 knock-out mice. Elimination of either pathway is embryonically lethal despite normal activity of the other pathway. PE can also be generated from a base-exchange reaction and by the acylation of PE. Cellular levels of PS and PE are tightly regulated by implementation of multiple compensatory mechanisms.
1. Biological Functions of Phosphatidylserine and Phosphatidylethanolamine in Mammalian Cells

Mammalian cell membranes contain more than a thousand 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, comprising 40 to 50% of total phospholipids. The second most abundant mammalian membrane phospholipid is phosphatidylethanolamine (PE) which comprises 20 to 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 to 10% of total phospholipids. The metabolic inter-relationships 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 to 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 grey matter, 22:6n-3 accounts for >36% of the fatty acyl species of PS (5-7). Since
22:6n-3 appears to be essential for normal development and functioning of the nervous system (6, 8-11) it is likely that PS plays an important role in the nervous system and in vision [reviewed in (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 PS decarboxylase (PSD) (section 3 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 uni-directional 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). Bi-directional 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, 27, 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 since nascent, intracellular very low density lipoproteins that are moving through the secretory pathway are highly enriched in PE compared to 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 (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).

2. *Phosphatidylserine 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 for making 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, that 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 correspondingly reduced (57, 58). Growth of the 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 chromosome 13 and 8, respectively. When PS synthase-2 activity was over-expressed in CHO cells the ethanolamine-exchange activity was increased 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 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, over-expression 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 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 syntheses? Do these syntheses perform distinct functions in cells or does the duplication confer merely a back-up
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 even 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 ubiquitously expressed 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, over-expression of PS synthase-1 activity in hepatoma cells increased the rate of incorporation of [3H]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 PS decarboxylase, and by reducing the formation of PE from the CDP-ethanolamine pathway (Fig. 1) (61). In contrast, an equivalent level of over-expression of PS synthase-2 activity in hepatoma cells did not increase the rate of incorporation of [3H]serine into PS or the conversion of PS to PE, or decrease the rate of synthesis of PE from CDP-ethanolamine (62). One potential explanation for the different responses of PS synthesis to 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 for ensuring 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 ~2-fold during apoptosis (68, 69) although there is no direct evidence that PS externalization is required for the initiation of apoptosis. However, over-expression of either PS synthase-1 or PS synthase-2 in CHO cells reduced the number of cells undergoing apoptosis in response to UV 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 concomitantly reduced (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 for providing the pool of PS that is externalized during apoptosis was investigated in CHO cell mutants that lacked either PS synthase-1 (58, 74), 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 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 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 Asn-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” (MAM) (82). MAM comprise a sub-domain 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 ~4-fold in MAM compared to the bulk of the ER (81, 82, 86). MAM have been
isolated from several types of mammalian cells (81, 86-89) as well as yeast (90, 91). A
specific marker protein for MAM in primary hepatocytes is phosphatidylethanolamine N-
methyltransferase-2 (82, 92). Increasing evidence indicates that juxtaposition of MAM
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 feed-back 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 (Arg-95) (56, 101) and PS synthase-2 (Arg-97) (102) that conferred resistance to feed-back 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 knock-out mice.** A powerful tool for understanding the function of specific genes in whole mammals is the generation of knock-out 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 sub-fertility.

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).

3. Phosphatidylethanolamine Synthesis

Mammalian cells employ two major pathways for PE biosynthesis: the CDP-ethanolamine pathway and the PS decarboxylation pathway (Figs. 1 and 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 and 2) (105). In yeast, this acyltransferase activity is highly enriched in MAM 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 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 PS decarboxylase (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 mono- or di-unsaturated 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 and parallels the CDP-choline pathway for phosphatidylcholine synthesis (122). Mammalian genes encoding the three enzymes of the CDP-ethanolamine pathway have been identified [reviewed in (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 approximately 20% of the pups died perinatally. Thus, it appears that the dual specificity ethanolamine/choline kinase is, at least partially, able to substitute for the ethanolamine-specific kinase in these knock-out 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) that 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 (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 ethanolamine-phosphotransferase 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 has recently been isolated (137). The mRNA encoding this protein is widely expressed in human tissues, including the cerebellum in the brain. Expression of the corresponding cDNA in *E. 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 alpha 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 MAM (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 unequivocally defined one likely possibility, for which there is some evidence, is that the transfer of PS from MAM 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 so far the gene involved has not yet been identified. In yeast, a ubiquitin ligase, Met30p, (91) is required for the import of newly-synthesized PS into mitochondria for decarboxylation [reviewed in (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 to 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 since lyso-PE is far more effective than ethanolamine in preventing mitochondrial defects in PSD-deficient yeast (106). In the yeast Saccharomyces cerevisiae PE is an essential phospholipid (152). PE made at non-mitochondrial 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 PS decarboxylase, 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 (for example, 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 (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 utilizes 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 post-translational 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 inter-relationships between these two phospholipids. Knock-out 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 implementation of compensatory mechanisms.
Figure legends

Figure 1. The metabolism of PS and PE in mammalian cells. Phosphatidylserine (PS) is made in elements of the endoplasmic reticulum 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 phosphatidylethanolamine (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 endoplasmic reticulum and nuclear envelope. Another major PE biosynthetic pathway occurs only in mitochondria and uses PS 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).

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