Defining the Importance of Phosphatidylserine Synthase 2 in Mice*

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Phosphatidylserine synthase 1 (Pss1) and phosphatidylserine synthase 2 (Pss2) produce phosphatidylserine by exchanging serine for the head groups of other phospholipids. Pss1 and Pss2 are structurally similar (~32% amino acid identity) but differ in their substrate specificities, with Pss1 using phosphatidylcholine for the serine exchange reaction and Pss2 using phosphatidylethanolamine. Whether Pss1 and Pss2 are both required for mammalian growth and development is not known, and no data exist on the relative contributions of the two enzymes to serine exchange activities in different tissues. To address those issues and also to define the cell type-specific expression of Pss2, we generated Pss2-deficient mice in which a β-galactosidase marker is expressed from Pss2 regulatory sequences. Histologic studies of Pss2-deficient mice revealed very high levels of β-galactosidase expression in Sertoli cells of the testis and high levels of expression in brown fat, neurons, and myometrium. The ability of testis extracts from Pss2-deficient mice to catalyze serine exchange was reduced by more than 95%; reductions of ~90% were noted in the brain and liver. However, we found no perturbations in the phospholipid content of any of these tissues. As judged by Northern blots, the expression of Pss1 was not up-regulated in Pss2-deficient cells and tissues. Testis weight was reduced in Pss2-deficient mice, and some of the Male mice were infertile. We conclude that Pss2 is responsible for the majority of serine exchange activity in in vitro assays, but a deficiency in this enzyme does not cause perturbations in phospholipid content or severe developmental abnormalities.

Phosphatidylserine is an aminophospholipid that constitutes 5–10% of mammalian membrane phospholipids (1). In mammals, phosphatidylserine is synthesized by a pair of enzymes, phosphatidylserine synthase 1 (Pss1)1,2 and phosphatidylserine synthase 2 (Pss2) (2–4), located primarily within the mitochondria-associated membrane fraction of the endoplasmic reticulum (5, 6). The two enzymes are structurally related, with 32% amino acid identity, and both are predicted to contain several transmembrane domains (2, 3, 6, 7). Pss1 and Pss2 generate phosphatidylserine by catalyzing the exchange of serine for the head group of another phospholipid, but the two enzymes differ in their substrate specificities. Pss1 uses phosphatidylcholine for the exchange reaction (8, 9), whereas Pss2 uses phosphatidylethanolamine (2–4, 6, 10). In vitro, Pss1 is capable of catalyzing the exchange of ethanolamine and choline in addition to serine; Pss2 is capable of catalyzing the exchange of ethanolamine but not choline. However, these ethanolamine and choline exchange reactions are not thought to be physiologically important for the in vivo synthesis of phosphatidylethanolamine (11) or phosphatidylcholine (12).

The physiologic “rationale” for the existence of two different phosphatidylserine synthases is unclear. No one knows whether mammalian growth and development require both enzymes, since no one has yet developed mice lacking either of the two genes. However, two groups have produced Chinese hamster ovary (CHO) cell lines lacking Pss1 by selecting for cells that required ethanolamine or phosphatidylserine for growth (8, 9). Extracts from the mutant CHO cells manifested a ~50% decrease in serine exchange activity, suggesting that Pss1 accounts for a significant portion of the serine exchange activity in that cell type. Pss1 deficiency did not have a significant impact on cell growth when the cells were grown in the presence of ethanolamine or phospholipids, but the cells grew slowly, and their phospholipid content was perturbed when the cells were grown in the absence of ethanolamine, phosphatidylserine, or phosphatidylethanolamine (7–9, 13).

Thus far, no one has developed cell lines that lack Pss2 expression, although a mutant CHO cell line that expressed reduced levels of Pss2 activity was generated (10). It is difficult to predict whether or not Pss2-deficient cells would be viable and healthy. On the one hand, one could argue that the exist-

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1 The abbreviations used are: Pss1, phosphatidylserine synthase 1; Pss2, phosphatidylserine synthase 2; CHO, Chinese hamster ovary; FSH, follicle-stimulating hormone; PBS, phosphate-buffered saline; β-gal, β-galactosidase; X-gal, 5-bromo-4-chloro-3-indolyl-β-d-galactopyranoside.

2 According to the International Committee on Standardized Genetic Nomenclature for Mice, the official gene symbols for phosphatidylserine synthase 1 and phosphatidylserine synthase 2 are Ptdss1 and Ptdss2, respectively. In this paper, we have used the names Pss1 and Pss2 because they are less cumbersome.
ence of Pss1 would make Pss2 expression superfluous. On the other hand, a large fraction of phosphatidylserine synthesis in CHO cells is due to Pss2, and it certainly would not be unreasonable to surmise that Pss2 would be crucial for cellular phospholipid homeostasis. It is also difficult to make a priori predictions about whether mice lacking Pss2 would be viable and, if so, whether there would be any pathology in tissues expressing high levels of the enzyme. Pss2 is expressed in a variety of different organs as judged by Northern blot analysis (6,7), but no information exists on which cell types express high levels of this gene. Finally, there are no biochemical data on the contribution of Pss2 to vital serine exchange activity in different mammalian tissues.

The purpose of this study was to define the physiologic importance of Pss2 in mammals. To address this issue, we produced Pss2-deficient mice in which the expression of a marker gene, β-galactosidase (β-gal), was driven by the regulatory elements of the Pss2 gene. The characterization of the Pss2-deficient mice allowed us to fill in a number of gaps in our knowledge of Pss2. First, we demonstrated that mice lacking Pss2 survive development and are viable, although males have small testes and occasionally testicular atrophy. We were able to define the impact of Pss2 deficiency on serine exchange activities in different tissues and were able to demonstrate, by β-gal staining, which cell types express high levels of Pss2. Finally, we investigated whether or not Pss2 deficiency perturbed the phospholipid composition of tissues and cells.

EXPERIMENTAL PROCEDURES

Generation of Pss2-deficient Mice—A mouse embryonic stem cell line (KST314, strain 129P2/OlaHsd) containing an insertional mutation in Pss2 was identified in a gene-trapping screen (14). The gene-trapping vector, pGKTMPs, was designed to interrupt genes that encode proteins with an N-terminal signal sequence and to create an in-frame fusion with the β-gal reporter gene (14). The embryonic stem cell line was used to generate male chimeric mice, which were bred with C57BL/6 mice to establish heterozygous (Pss2+/−) and homozygous (Pss2−/−) knockout mice. Mice were genotyped by quantifying neomycin phosphotransferase II (neo) gene dosage in genomic DNA with a quantitative PCR assay (described on the BayGenomics Web site at baygenomics.ucsf.edu/protocols). Genotyping was also performed by quantifying neo gene dosage with Southern blots; for these studies, BamHI-digested genomic DNA was hybridized with pGKTMPs that had been linearized with HinDIII. All mice described here had a mixed genetic background, including 50% C57BL/6 and 50% C3H/HeN mice. These mice were weaned at 21 days of age, housed in a barrier facility with a 12-h light/dark cycle, and fed a chow diet containing 4.5% fat (Ralston Purina, St. Louis, MO).

Pss2−/−, Pss2+/−, and wild-type (Pss2+/+) embryonic fibroblasts were prepared from 13.5-day mouse embryos (15) and immortalized by serial passaging (15).

Northern Blot Analysis—Sites of Pss2 and Pss1 expression were determined with mouse multiple-tissue poly(A)+ RNA blots (CLONTECH, Palo Alto, CA). The protein-coding sequence of the Pss1 cDNA was amplified from a mouse liver cDNA library (CLONTECH) with oligonucleotides 5′-ATGCGCTGCTGCGGAGAACGAG-3′ and 5′-CACAGGAGGATAGAGTTTACAC-3′ and cloned into pCRII (Invitrogen). A 1.1-kb Pss1 cDNA probe was produced by removing the insert with EcoRI. A 1.4-kb Pss2 cDNA was cloned into pCRII (7); the insert was released by EcoRI digestion. Probes were labeled with [32P]dCTP by random hexamer priming. The Northern blots were exposed to x-ray film at −80°C for 2 h at −80°C.

Northern blots were also produced with total RNA isolated from brain, liver, and testis from Pss2+/+ and Pss2−/− mice. RNA was isolated with the Tri Reagent RNA isolation kit (T9434; Sigma). Total RNA (20 μg) was separated by electrophoresis on a 1% agarose/formaldehyde gel and then transferred to a Nytran SuPerCharge membrane (Schleicher & Schuell) and hybridized with the 1.4-kb Pss2 cDNA probe. The blots were washed at high stringency in a solution containing 0.1% SDS, 2× SSC, and 0.1× SSC for 2 h at 68°C. The Northern blots were also produced with total RNA isolated from heart, lung, spleen, kidney, liver, and testis from Pss2−/− mice. RNA was isolated with the Tri Reagent RNA isolation kit (T9434; Sigma). Total RNA (20 μg) was separated by electrophoresis on a 1% agarose/formaldehyde gel and then transferred to a Nytran SuPerCharge membrane (Schleicher & Schuell) and hybridized with the 1.4-kb Pss2 cDNA probe. The blots were washed at high stringency in a solution containing 0.1% SDS, 2× SSC, and 0.1× SSC for 2 h at 68°C.

Assays of Phosphatidylserine Synthase Activity—Tissue samples (~100 mg) were homogenized with a Polytron homogenizer in a buffer containing 50 mM Tris-HCl (pH 7.4), 5 mM MgCl2, 1.0 mM EDTA, and 100 mM NaCl. The tissue homogenate was centrifuged for 5 min at 600 × g, and the supernatant fluid was used for measurement of serine exchange activity. The reaction mixture (200 μl) contained 160 μl of tissue sample with 300 μg of protein, 20 μl of a buffer (100 mM CaCl2, 40 mM hydroxyamine, and 250 mM HEPEs, pH 7.4), and 20 μl of the radiolabel (a 14C)serine solution, an 14C)ethanolamine solution, or a 14C)choline solution. The 14C)serine solution contained 10 μl of 14C)serine (200 μCi; Sigma). The 14C)ethanolamine solution contained 10 μl of 14C)ethanolamine (CFA329; Amersham; 5 μCi/μl in a volume of 250 μl) and 990 μl of 0.2 μM unlabeled ethanolamine (E-9508; Sigma). The 14C)choline solution contained 4 μl of 14C)choline (CFA329; Amersham; 5 μCi/μl in a volume of 250 μl) and 990 μl of 0.2 mM unlabeled choline (C-1879; Sigma). The reaction was allowed to proceed for 20 min at 37°C and was terminated by adding 5 ml of chloroform/methanol (2:1, v/v). A total of 1.5 ml of water was then added to each tube, and the tubes were centrifuged at 1,000 × g for 5 min. The upper phase was aspirated and discarded. The lower phase was washed three times with 2.0 ml of methanol/water (1:1). The phospholipid products were extracted by the method of Bligh and Dyer (17), and radioactivity was measured.

Metabolic Labeling of Phosphatidylserine in Embryonic Fibroblasts—Immortalized fibroblasts from Pss2−/− and Pss2+/− embryos were grown to 80–90% confluence in 80-mm dishes in the presence of 15% fetal bovine serum. The cells were then incubated for up to 6 h with 3 μCi/ml [3H]serine ([3H]Pss2, and wild-type ([3H]Pss2−/−) knockout mice. Mice were genotyped by quantifying neomycin phosphotransferase II (neo) gene dosage in genomic DNA with a quantitative PCR assay (described on the BayGenomics Web site at baygenomics.ucsf.edu/protocols). Genotyping was also performed by quantifying neo gene dosage with Southern blots; for these studies, BamHI-digested genomic DNA was hybridized with pGKTMPs that had been linearized with HindIII. All mice described here had a mixed genetic background, including 50% C57BL/6 and 50% C3H/HeN mice. These mice were weaned at 21 days of age, housed in a barrier facility with a 12-h light/dark cycle, and fed a chow diet containing 4.5% fat (Ralston Purina, St. Louis, MO).

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second centrifugation was subjected to ultracentrifugation for 1 h at 100,000 × g; the resultant pellet was designated as microsomes. Lipids were extracted from the microsomes (17) and separated by thin layer chromatography in the solvent system chloroform/methanol/acetic acid/formic acid/water (70:30:12:4:2, v/v/v/v/v). Phospholipids were identified by exposure to iodine vapor and comparison with standards. Bands corresponding to phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol were scraped from the plate, and the amount of each phospholipid (nmol/mg of protein) was determined by measurement of lipid phosphorus (19).

RESULTS

Generation of Pss2 Knockout Mice—Pss1 and Pss2 are both expressed in a broad and overlapping group of organs, as judged by multiple-tissue Northern blots (Fig. 1, A and B). Pss2 is expressed at particularly high levels in the testis, but Pss1 is also expressed there. Pss1 is highly expressed in liver and heart, but Pss2 is also expressed in those tissues.

To determine whether normal expression levels of both of the phosphatidylserine synthases are required for mammalian development and for viability of adult mice, we generated Pss2 knockout mice with an embryonic stem cell line containing an insertional mutation in intron 2 of Pss2. The mutation results in the production of a fusion transcript containing Pss2 sequences encoding the first 72 amino acids of the enzyme (including the first transmembrane segment) spliced to the CD4 transmembrane domain and β-geo sequences from the gene trap vector (a fusion between Escherichia coli lacZ [encoding β-gal] and neo) (20, 21). The production of the Pss2-β-geo fusion transcript is under the control of the Pss2 regulatory sequences.

Pss2−/− mice develop normally, appear healthy, and grow at the same rate as littermate control mice. The plasma cholesterol, triglyceride, and phospholipid levels were no different than in wild-type littermates (data not shown). Female Pss2−/− mice exhibited normal fertility, as did the majority of the males, but ~10% of the male Pss2−/− mice (n = 33 examined) were infertile or subfertile. The absence of a severe phenotype was not due to “leakiness” of the insertional mutation (e.g. production of a normal Pss2 transcript from the mutant allele). No normal Pss2 transcript (2.4 kb) was detectable in knockout mice (Fig. 1C). As expected, a ~10-kb Pss2-β-geo fusion transcript could be observed on a Northern blot hybridized with a neo probe (Fig. 1D).

The knockout of Pss2 did not result in increased Pss1 mRNA expression, either in fibroblasts cultured from Pss2−/− embryos (Fig. 1E) or in the testis or brain of adult Pss2−/− mice (Fig. 1F).

Phosphatidylserine Synthase Activity Is Reduced in Pss2-deficient Mice—To explore the contribution of Pss2 to total phosphatidylserine synthase activity in mammalian tissues, we measured the ability of tissue extracts to catalyze the exchange of radiolabeled serine, radiolabeled ethanolamine, and radiolabeled choline into phospholipids. Serine exchange activity was ~95% lower in extracts from Pss2−/− testes than in extracts from Pss2+/+ testes (Fig. 2A). Serine exchange was also reduced substantially in the brain, heart, and liver of Pss2−/− mice, although to a lesser degree than in the testes (Fig. 2A). The ability of tissue extracts from Pss2−/− mice to exchange ethanolamine was also markedly reduced (Fig. 2B). Interestingly, choline exchange, which is indicative of Pss1 activity, was increased by about 2-fold in Pss2−/− tissues.
Base exchange activities in tissues from Pss2/H11001/H11002 mice were intermediate between those of Pss2/H11001/H11001 and Pss2/H11002/H11002 mice (data not shown).

Phospholipid Content of Membranes from Pss2/H11001/H11001 and Pss2/H11002/H11002 Tissues—Pss1 deficiency in CHO cells reduced phosphatidylserine synthase activity, and phospholipid composition of the mutant cells was perturbed when the cells were grown in ethanolamine- or phospholipid-depleted medium (6, 8, 9, 13). To determine whether Pss2 deficiency affected tissue phospholipid composition, we measured the phospholipid content of the liver, brain, and testis of Pss2/H11002/H11002 mice and littermate Pss2/H11001/H11001 controls. No significant differences were observed (Fig. 3). We also measured the phospholipid content of microsomes from the liver and testis of Pss2/−/− and Pss2/+ mice. Again, no significant differences were observed (Fig. 4).

We considered the possibility that the normal phospholipid

**Fig. 2.** Base exchange activities of tissue extracts from Pss2/+ and Pss2/− mice with radiolabeled serine (A), ethanolamine (B), and choline (C). The ability of tissue membrane fractions to exchange [14C]serine, [14C]ethanolamine, and [14C]choline onto phospholipids was measured. Values are means ± S.D. of analyses of the brain, heart, liver, and testis from five separate Pss2/+ (black bars) and Pss2/− (open bars) mice. *, p < 0.05; **, p < 0.01; ***, p < 0.001 by t test.

**Fig. 3.** Phospholipid composition of homogenates of liver (A), brain (B), and testis (C) of Pss2/+ and Pss2/− mice. Black bars, phosphatidylserine; white bars, phosphatidylethanolamine; gray bars, phosphatidylycholine; hatched bars, sphingomyelin. Values are means ± S.D. of independent measurements of tissues from five separate mice of each genotype.

**Fig. 2C.** Base exchange activities in tissues from Pss2/+ mice were intermediate between those of Pss2/+ and Pss2/− mice (data not shown).
composition of Pss2−/− tissues might have been due to an equilibration of phospholipids at the whole-animal level, perhaps as a result of the delivery of phospholipids to tissues by plasma lipoproteins. To test that possibility, we examined serum exchange activity and phospholipid content of Pss2+/+ and Pss2−/− fibroblasts grown for 72 h in lipoprotein-deficient serum. Serine exchange in Pss2−/− fibroblasts was reduced by ~90% (Fig. 5A); however, no significant perturbation in cellular phospholipid composition was noted (Fig. 5B). To test whether the reduced serum exchange activity in extracts from Pss2−/− fibroblasts corresponded to a decrease in phosphatidylserine synthesis in intact cells, we determined the incorporation of radiolabeled serine into phosphatidylserine. Phosphatidylserine synthesis was reduced in Pss2−/− fibroblasts (Fig. 5C).

Cell Type-specific Expression of Pss2—The cell types responsible for high levels of Pss2 expression are unknown. To address this issue, we examined β-gal expression (reflecting sites of Pss2 expression) in 20-day mouse Pss2+/− embryos and in adult Pss2−/− mice. In embryos, the highest levels of β-gal expression were in brown adipose tissue over the dorsal thoracic cage (Fig. 6A, arrowheads) and in peripheral nerves (Fig. 6A, arrows). As expected, the brown adipose tissue stained intensely with Oil Red O (Fig. 6B). In adult mice, β-gal staining was intense in the brain, uterus, and testis. In the brain, β-gal expression was confined to neurons and was particularly prominent in the Purkinje cells of the cerebellum (Fig. 6, C and D) and in pyramidal neurons in the CA1 region of the hippocampus (Fig. 6E). High levels of β-gal expression were also observed in the myometrium of the uterus (Fig. 6F).

In tests of 4–6-month-old Pss2+/− mice (n = 25), we observed two histologic patterns. In the majority of the Pss2−/− mice (n = 22), testis histology appeared normal by light microscopy, with small numbers of Leydig cells in the interstitium, Sertoli cells surrounding the spermatids, and abundant spermatagonia and maturing spermatocytes (Fig. 7A). In the remaining three Pss2−/− mice, the testes were severely atrophic (~30% of normal size), and histologic examination revealed hyperplasia of Leydig cells and contracted spermatogenic ducts with a thick layer of Sertoli cells and no spermatocytes (Fig. 7B). We never observed atrophic testes in Pss2+/− (n = 9) or Pss2−/− littermate controls (n = 27). Regardless of whether the histology of Pss2−/− mice was normal or abnormal, β-gal staining of Sertoli cells was intense (Fig. 7, A and B). Beta-Gal staining was detectable in Sertoli cell projections adjacent to spermatocytes, but no staining of spermatogonia or spermatocytes was observed. Higher power images revealed punctate β-gal staining within Leydig cells, but the staining intensity was invariably lower than in Sertoli cells.

The finding of testis atrophy in 3 of 25 Pss2−/− mice but in none of the controls suggested that Pss2 deficiency might cause overt testis pathology but with incomplete penetrance. If this were the case, we hypothesized that a more thorough examination of male Pss2−/− mice might uncover subtle abnormalities in testis size or function, even in animals with normal testis histology. To test this possibility, we compared testis weights in male Pss2−/− mice (n = 30, all with normal testis histology) and in littermate Pss2+/+ mice (n = 27). These studies revealed a small (~13%) but highly significant (p < 0.01) reduction in testis weight in Pss2−/− mice (Fig. 8A).

Sertoli cells normally produce inhibin B, which negatively regulates serum FSH levels (22). Thus, Sertoli cell dysfunction leads to low serum inhibit B levels and high serum FSH levels (22). We suspected that subtle Sertoli cell dysfunction in Pss2−/− mice might be accompanied by increased serum FSH levels. Indeed, this was the case; serum FSH levels in Pss2−/− mice were significantly higher than in littermate controls (p < 0.01) (Fig. 8B).

**DISCUSSION**

Over the past 10 years, a series of biochemical and genetic studies have established that mammals have two different enzymes for generating phosphatidylserine, Pss1 and Pss2 (2–4, 7). These two enzymes have significant sequence similarity but differ in their substrate specificities. In this study, we generated Pss2-deficient mice and used those mice and derivative cell lines to address a number of mysteries surrounding the mammalian phosphatidylserine-synthesizing enzymes. Our studies have added important new information to the field. First, it seems clear that both enzymes are not required for development. The observations that homozygous Pss2-deficient mice are viable, grow normally, and maintain normal tissue phospholipid compositions indicate that Pss2 is largely dispensable for development, at least under laboratory conditions. Second, we defined, by β-gal staining, the cell types that express Pss2 at high levels. The highest levels of Pss2 expression were located in the brown fat during development and in the Sertoli cells of the testis in adult mice. Third, we demonstrated that Pss2 accounts for the majority of serine exchange activity in mammalian tissues. Fourth, we demonstrated that Pss2 is not required for the maintenance of normal phosphatidylserine levels in cultured fibroblasts, even when the cells are deprived of ethanolamine and exogenous phospholipids.

Testicular atrophy was noted in ~10% of male Pss2−/− mice, and male mice lacking overt testicular atrophy had smaller testes than littermate controls. We suspect that these testes phenotypes could relate to a borderline capacity to synthesize phosphatidylserine in Sertoli cells, which provide nutritional support to the germ cells within the spermatogenic ducts (23, 24). Aside from the reduced testis weight, borderline Sertoli cell function was also suggested by the increased serum FSH levels. Interestingly, we could not document any signifi-
cant perturbations in phospholipid composition in extracts of whole testes, although serine exchange activity was reduced by >95%. It is conceivable that phosphatidylserine levels could be reduced in a subset of the cells in the testes, such as the Sertoli cells, but we suspect that any such reductions would be slight and might well be buried within the variation inherent in measuring phospholipids.

Extracts from $Pss2$-deficient tissues manifested an unequivocal increase in choline exchange activity. Choline exchange is mediated by $Pss1$ (4, 6, 8). A simple explanation for the finding would have been an increase in $Pss1$ expression in response to the deficiency in $Pss2$. However, as judged by Northern blots, $Pss1$ mRNA expression was entirely normal, both in mouse tissues and in cultured fibroblasts (Fig. 1, E and F). We favor an alternative explanation, that the increased level of choline exchange in $Pss2$−/− tissues reflects a posttranscriptional increase in $Pss1$ activity. Kuge et al. (25) demonstrated that $Pss1$ activity is reduced by increased levels of phosphatidylserine in membranes, and they even identified a specific amino acid within $Pss1$ (Arg-95) that is crucial for the end product regulation of the enzyme. Thus, $Pss2$ deficiency could result in low levels of phosphatidylserine in some regulatory pool (i.e. membranes in the vicinity of $Pss1$), resulting in increased $Pss1$ enzymatic activity and thereby explaining the higher levels of choline exchange activity.

Serine exchange activity was reduced by −90% in $Pss2$-deficient fibroblasts, but the phospholipid composition was normal, even when the fibroblasts were grown in ethanolamine-
Decreasing the rate of phosphatidylserine synthesis could result in a lower rate of phosphatidylserine and/or phosphatidylethanolamine degradation, facilitating the maintenance of constant phospholipid levels.

The metabolic labeling experiments in fibroblasts (Fig. 5C) suggested that the synthesis of phosphatidylserine is reduced by Pss2 deficiency, in keeping with the reduction in serine exchange activity. However, caution is advised in interpreting those fibroblast experiments. The cell lines used for those experiments were derived from different embryos (which were not on an inbred background) and had undergone independent immortalization events. In interpreting this experiment, one must consider the possibility that extraneous genetic differences, aside from the Pss2 mutation, could have had an indirect effect on phosphatidylserine synthesis rates. In the future, we believe that it will be essential to perform a comprehensive analysis of phosphatidylserine biosynthesis and turnover rates in multiple independent fibroblast cell lines and in multiple tissues from Pss2-deficient mice.

Our current observations contrast with earlier results with Pss1-deficient CHO cells (8, 9). In the CHO cells, serine exchange activity was reduced by ~50% (using the same assay), but there were highly significant decreases in phosphatidylserine and phosphatidylethanolamine levels when the cells were grown in ethanolamine-free and phospholipid-depleted media (7–9, 13). Why is the phenotype of Pss1 deficiency in CHO cells so different? We speculate that the differences might be explained by the different substrate specificities of the two enzymes. We suspect that Pss2 deficiency is well tolerated because Pss1 is still capable of generating phosphatidylserine from phosphatidylcholine. Even when Pss2-deficient cells are deprived of ethanolamine, phosphatidylserine continues to be produced from phosphatidylcholine, and phosphatidylethanolamine can still be generated by decarboxylation of phosphatidylserine. In contrast, in the setting of Pss1 deficiency, Pss2 can generate phosphatidylserine, but only if phosphatidylethanolamine is available for the exchange reaction. In the absence of exogenous ethanolamine, when phosphatidylethanolamine cannot be generated from the CDP-ethanolamine pathway, there is no way to synthesize significant amounts of phosphatidylserine or phosphatidylethanolamine. In the setting of Pss1 deficiency, phosphatidylethanolamine can only be generated from phosphatidylserine, and phosphatidylserine can only be generated from phosphatidylethanolamine. Thus, Pss1-deficient cells are dependent on exogenous ethanolamine to replenish their phosphatidylserine and phosphatidylethanolamine stores.

The availability of Pss2-deficient mice will make it possible to address a host of issues in the future. For example, we are intrigued by the high levels of Pss2 expression in neurons within the brain. Large amounts of phosphatidylserine are normally imported into mitochondria and then converted to phosphatidylethanolamine (26–29). It would be interesting to determine whether the neurons of Pss2-deficient mice might be more susceptible to injury in response to metabolic conditions that limit the production of energy from mitochondria (e.g., hypoxemia or hypoglycemia). The current studies also raise several other issues for future experimentation. High on the list is how mice would respond to a diet containing low levels of ethanolamine. We would also be interested in whether a single Pss1 knockout allele would elicit pathologic findings in Pss2−/− mice. Answers to these questions will probably emerge over the next few years.
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