In mammalian cells, phosphatidylserine is synthesized by two different enzymes, phosphatidylserine synthase (PSS)-1 and -2, via a base exchange reaction in which the head group of a phospholipid (phosphatidylcholine or phosphatidylethanolamine) is replaced by l-serine. Since the amino acid sequences of PSS1 and PSS2 are only ~30% identical, it is likely that they are encoded by different genes. We have screened a murine liver genomic DNA library, included in bacterial artificial chromosomes, with full-length murine PSS1 cDNA and isolated a clone containing the majority of the PSS1 gene. This gene spans ~35 kilobases and contains 13 exons and 12 introns. The sizes of the exons range from 44 to 1035 base pairs. The gene was localized to chromosome 15 in region B-C1. According to reverse transcriptase-mediated polymerase chain reaction, PSS1 and PSS2 mRNAs were expressed in all murine tissues examined. The mRNA encoding PSS1 was most abundant in kidney, brain, and liver, whereas PSS2 mRNA was most highly expressed in testis. In general agreement with the levels of mRNA expression, the choline exchange activity (contributed by PSS1, but not PSS2) was highest in brain, whereas serine and ethanolamine exchange activities were highest in testis and kidney. The transcriptional initiation site for PSS1 was identified 111 base pairs upstream of the ATG specifying the start of translation. The putative 5′-proximal promoter region of the gene contained no TATA or CAAT box, but did have a high GC content. Isolation of the murine PSS1 gene is a step toward generation of genetically modified mouse models that will help to understand the functions of PSS1 and PSS2 in animal biology.

Phosphatidylserine (PtdSer) is an important amino phospholipid that accounts for 5–10% of animal cell membrane phospholipids. In addition to a presumed structural role in membranes, PtdSer is an activator of protein kinase C (1) and is involved in progression of the blood coagulation cascade (2, 3). PtdSer exposure on the external leaflet of the plasma membrane, via an amino-phospholipid translocase (4, 5) and scramblase (6–8), is also a signal for recognition and removal of apoptotic cells by macrophages (5, 9). In mammalian cells, PtdSer is synthesized by a calcium-dependent base exchange reaction catalyzed by PtdSer synthase (PSS) activity that exchanges l-serine for the polar head group of phosphatidylcholine (PtdCho) and phosphatidylethanolamine (PtdEtn) (10, 11). The pathway for PtdSer synthesis in mammals is different from that in bacteria (12) and Saccharomyces cerevisiae (13, 14), in both of which PtdSer is synthesized through reaction of l-serine with CDP-diacylglycerol. Interestingly, plants make PtdSer from both the CDP-diacylglycerol pathway (15) and a base exchange reaction (16).

The existence of two mammalian PSSs, PSS1 and PSS2, was deduced from studies with mutant Chinese hamster ovary cell lines (17–19). PSS1 and PSS2 differ in their phospholipid substrate specificities (17, 18), but the reason why mammalian cells possess two distinct PSSs is not clear (20). In intact cells, PSS1 uses PtdCho as donor of the phosphatidyl group, whereas PSS2 uses PtdEtn (21). In vitro enzymatic assays, however, PSS1 uses both PtdCho and PtdEtn. In Chinese hamster ovary cells and rat liver, serine exchange activity is associated with the endoplasmic reticulum (22, 23) and is enriched 2–4-fold in an endoplasmic reticulum subfraction, the mitochondrion-associated membranes, compared with the bulk of the endoplasmic reticulum (24–27). Mitochondrion-associated membranes have been proposed to consist of a specialized domain of the endoplasmic reticulum that is juxtaposed with mitochondria, and mitochondrial-associated membranes have been proposed to mediate the import of newly synthesized PtdSer into mitochondria (24, 25, 28, 29). Using antibodies raised against PSS1 and PSS2 as well as Myc-tagged murine PSS1 and PSS2 expressed in rat hepatoma cells, we have recently shown that these two proteins are localized almost exclusively to mitochondrion-associated membranes (26).

The cDNAs encoding PSS1 and PSS2 from murine liver (30, 31) and CHO-K1 cells (18, 32) have been cloned and expressed. Since the predicted amino acid sequences of PSS1 and PSS2 are only ~30% identical, with no long continuous stretches of homology (18, 31), PSS1 and PSS2 appear to be encoded by different genes. We have expressed murine PSS1 and PSS2 cDNAs in McArdle rat hepatoma cells and M.9.1.1 cells (a Chinese hamster ovary cell line lacking PSS1 (19)) (30). When the synthesis of PtdSer was increased by expression of murine PSS1 in hepatoma cells, the cellular content of PtdSer and PtdEtn remained unchanged, whereas the production of PtdEtn via PtdSer decarboxylation increased, and PtdEtn synthesis via the CDP-ethanolamine pathway was reciprocally decreased (30). These data suggest that the rates of synthesis...
and degradation of PtdSer and PtdEtn are tightly regulated.

We now report the isolation of the murine PSS1 gene. A murine genomic DNA library, contained in bacterial artificial chromosomes (BAC), was screened with full-length cDNA encoding murine PSS1. Examination of positive BAC clones showed that the PSS1 gene resides on murine chromosome 13 in region B-C1 and consists of 13 exons and 12 introns. We also demonstrate, using reverse transcriptase-mediated PCR and enzymatic assays, that PSS1 and PSS2 are widely, but differ-

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EXPERIMENTAL PROCEDURES

Materials—Restriction endonucleases, the random primer labeling kit, and the end labeling kit were obtained from Life Technologies, Inc. (Burlington, Ontario, Canada). The radioisotopes ([α-32P]CTP and [γ-32P]ATP were from Amersham Pharmacia Biotech (Quebec, Cana-
dada). Accurase long-range polymerase was from DNamp Ltd. (Farnbor-
ough United Kingdom) and Ex-Taq and Rec-Taq polymerases were
from Takara Biomedicals, Inc. (Panvera, Madison, WI). The TOPO-TA cloning kit was from Invitrogen (Mississauga, Ontario). All other re-
agents were obtained from Sigma (Oakville, Ontario) or Fisher (Ne-
pean, Ontario).

DNA Isolation and Southern Analysis—BAC DNA was isolated using a QIAGEN midiprep kit (tip 100), and plasmid DNA was isolated
with a Wizard miniprep or maxiprep kit (Promega, Madison, WI). For South-
a QIAGEN midiprep kit (tip 100), and plasmid DNA was isolated
with a Wizard miniprep or maxiprep kit (Promega, Madison, WI). For South-
internal probe (276AS; see Table I). The same PCR conditions were used with two primers specific for PSS2 (1S, TGGAGTCACACAAGC-CAAGAG; and 2AS, GTAGTTGGATAGTCTTCAAGG). The identity of the product was confirmed by Southern blot analysis using oligonucleotide 3S (GGACAGCTTGAGAAGCTTTGT) as a probe. Cyclophin was used as an internal control (95 °C for 2 min; 74 °C for 1 min, 61 °C for 30 s, and 74 °C for 30 s and an extension step at 74 °C for 3 min).

**Identification of the Transcriptional Initiation Site Using 5′-RACE—**

Total RNA was prepared from liver samples of 129/J mice using Trizol according to the manufacturer's instructions, and RNA quality was evaluated by performing electrophoresis on 1% agarose gels stained with ethidium bromide. Total RNA was reverse-transcribed using a 22-mer primer (913AS; see Table I) and Superscript II reverse transcriptase as described above. The cDNA was washed using the Prep-A-gene DNA purification kit (Bio-Rad) and then tailed using dATP and recombinant terminal deoxynucleotidyltransferase (Life Technologies, Inc.). Briefly, 4 μl of ATP (1 mM), 5 μl of buffer, and 1 μl of enzyme were added to the cDNA and incubated at 37 °C for 15 min. The reaction was terminated by incubation at 70 °C for 10 min. The incubation mixture was diluted to 500 μl with Tris/EDTA buffer (pH 8.0). PCR was performed on the reverse-transcribed tailed products using an antisense primer (396AS; see Table I) and a sense primer (Race dT, GACTC-GAGTGCAGACGTGCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT) and Superscript II reverse transcriptase. The reaction mixture was diluted to 1 ml with 10 mM Tris buffer (pH 8.0) containing 1 mM EDTA. PCR was performed using adapter primer Ad68 and primer 620S (see Table I) with the following program: 95 °C for 5 min; 55 °C for 3 min; 72 °C for 40 min; 30 cycles of 95 °C for 45 s, 55 °C for 45 s, and 72 °C for 45 s; and a final step at 72 °C for 10 min. Southern blot analysis was performed on the reaction products using radiolabeled internal probe (85S; see Table I). Hybridizing fragments were subcloned into pCR2.1 using the TOPO-TA kit and sequenced. The sequence was compared with that of murine PSS1 cDNA.

**3′-Untranslated Region of PSS1 cDNA and Its Location on the PSS1 Gene—**

Total RNA was prepared from 129/J mouse liver using Trizol. RNA (2 μg) was reverse-transcribed for 3 h at 42 °C using adapter primer Ad68 dT (GGGCACGGCTGACGTAGACAAAA) specific for the poly(A) tail. The program used for the PCR was as follows: 95 °C for 2 min; 35 cycles of 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min; 72 °C for 2 min; and an extension step at 72 °C for 8 min. Southern blot analysis was performed on products of the reaction using a radiolabeled probe (85S; see Table I). Hybridizing fragments were subcloned into pCR2.1 using the TOPO-TA kit and sequenced. The sequence was compared with that of murine PSS1 cDNA.

**Analysis of the Putative Proximal Promoter—**
The 5′-sequence proximal to the transcriptional initiation site was obtained by “gene walking” using primers 1U, 2U, 3U, and 4U (see Table I) for sequencing directly from 5 μg of BAC DNA. The sequence of the putative promoter region was evaluated using the Transfac search engine.

**Chromosome Localization—**
The chromosome location of the murine PSS1 gene was determined by DNA Biotech Inc. at the University of Toronto (Toronto, Canada). Lymphocytes were isolated from mouse spleen and cultured at 37 °C in RPMI 1640 medium supplemented with 15% fetal calf serum, 3 mg/ml concanavalin A, 10 μg/ml polyinosinic acid, and 5 × 10⁻⁶ M β-mercaptoethanol. After 44 h, cultured lymphocytes were treated for 14 h with 0.18 mg/ml 5-bromo-2′-deoxyuridine. The synchronized cells were washed and re-cultured at 37 °C for 4 h in minimal essential medium containing thiamine (2.5 μg/ml). Chromosome identification slides were prepared by hypotonic treatment, fixation, and air drying. For the DNA probe, we used a pure preparation of BAC clone 595 F12 isolated using a QIAGEN midiprep kit (tip 100). The DNA probe was biotinylated with dATP using the BioNick labeling kit (15 °C, 1 h; Life Technologies, Inc.) (33). The procedure for FISH detection was as described previously (33, 34). Briefly, slides were baked at 55 °C for 1 h and then treated with RNase A. The material on the slides was denatured with 70% formamide in 2 × SSC for 2 min at 70 °C, followed by dehydration with absolute ethanol. Probes were denatured at 75 °C for 5 min in a hybridization mixture consisting of 50% formamide and 10% dextran sulfate and then prehybridized for 15 min at 37 °C. After an overnight hybridization reaction with the probes, the slides were washed at various temperatures, dehydrated, and amplified (33). FISH signals and the DAPI banding patterns were recorded separately, and the assignment of the FISH mapping data to chromosomal bands was achieved by superimposing FISH signals with DAPI-banded chromosomes (34).

**Measurement of PSS Enzymatic Activity—**
PSS activity was determined as previously described (24). Briefly, frozen tissues were homogenized in HEPES buffer (pH 7.5) containing 0.25% saponin. Homoge-
nates were centrifuged for 10 min at 1000 × g to pellet nuclei and cellular debris, and PSS activity was measured in the supernatant. Typically, 50–100 μg of protein were incubated with [3-3H]serine (50 μCi/μmol, 0.4 mCi), [1-3H]ethanolamine (20 μCi/μmol, 0.2 mCi), or [methyl-3H]choline (50 μCi/μmol, 0.2 mCi) in 25 mM HEPES buffer (pH 7.4) containing 4 mM hydroxylamine and 10 mM CaCl₂ in a final volume of 200 μl for 20 min at 37 °C. The reaction was terminated by addition of 5 ml of chloroform/methanol (2:1, v/v). Water (1.5 ml) was added for phase separation, after which the lower phase was washed three times with 1.5 ml of methanol/water (1:1, v/v), and radioactivity was measured.

RESULTS AND DISCUSSION

Cloning and Exon-Intron Organization of the Murine PSS1 Gene—Five BAC clones were isolated from a 129/J murine genomic library by PCR screening and Southern blot analysis using radiolabeled exon-specific probes and radiolabeled full-length PSS1 cDNA. One of these genomic clones, 595 F12, contained the majority of the murine PSS1 gene. However, this clone lacked 437 base pairs at the 3'-end of the PSS1 cDNA. None of the other four clones contained the entire 3'-end of the cDNA. The integrity and identity of the cloned PSS1 gene were confirmed by comparison of the restriction enzyme fragmentation patterns of genomic DNA and the BAC clone (data not shown). One restriction fragment derived from genomic DNA was not the same size as any of those derived from the BAC clone. We confirmed that this fragment corresponded to the 3'-end of the PSS1 gene by performing PCR amplification using oligonucleotide primers (1920S and 2312AS; Table I) specific for the 3'-end of murine PSS1 cDNA, with genomic DNA and full-length PSS1 cDNA as templates. Although clone 595 F12 lacked 437 base pairs at the 3'-end of the cDNA, this portion of the gene did not appear to contain any introns since (i) the PCR product obtained using murine liver genomic DNA as a template was the same size as the PCR product that would be expected from PSS1 cDNA, and (ii) the sequence of the human PSS1 gene obtained from the NCBI Database (accession number AC068091, 966284529-3551-12509) did not reveal any introns in this region of the human gene. Radiolabeled full-length cDNA encoding murine PSS2 was also used in Southern blot analysis to confirm that the clones with which PSS1 cDNA hybridized did not contain any introns. These results demonstrate that murine PSS1 and PSS2 are encoded by different genes. On the basis of these results, clone 595 F12 was used to study the organization of the PSS1 gene.

A combination of Southern blotting and sequencing using specific primers identified the exonic sequences and facilitated determination of exon and intron sizes as well as sequences at the exon-intron splice junctions (Fig. 1 and Tables II and III). The PSS1 gene is ~35 kb in length, which is ~15 times longer than the PSS1 cDNA. The gene is composed of 13 exons interrupted by 12 introns (Fig. 1 and Table II). Exon 1 contains the ATG codon of the translational start site (Fig. 1A). We estimated that the sizes of introns VII and XI are 2.3 and 2.2 kb, respectively. We established and sequenced the exon-intron boundaries (Table III). The boundary sequences at the 5’- and 3’-ends of all the introns, except intron VII, are GT and AG, respectively. These are consensus sequences for pre-mRNA splicing donor and acceptor sites (35). Clone 595 F12 was digested separately with BamHI, EcoRI, HindIII, XbaI, and KpnI, and the resulting fragments were analyzed by Southern blotting with probes specific for individual exons (Fig. 1B and Table I). Using this strategy, we isolated several overlapping clones, which were subcloned into Bluescript. Clone 1 (BamHI, 5.2 kb) contains the first exon with 900 bp of the first intron; clone 2 (XbaI, 2.3 kb) contains the first exon with 700 bp of the first intron; and clone 3 (KpnI, 5.5 kb) contains the first exon with 4.1 kb of the first intron (Fig. 1B). The remainder of the gene was similarly mapped. Some of these clones will be used for targeted disruption of the murine PSS1 gene.

Identification of the Transcriptional Initiation Site—The transcriptional initiation site of the PSS1 gene was identified by 5’-RACE PCR. Total RNA was isolated from 129/J mouse liver and reverse-transcribed using primer 913AS (Table I) with Superscript reverse transcriptase. The resulting cDNA product was tailed with dATP. This template was amplified by PCR with the primer pair Race dT and 396AS (Table I and Fig. 2A). A single PCR product of 420 bp was generated (Fig. 2B, upper). Southern blot analysis of this PCR product, which hybridized with a probe corresponding to the 5’-end of PSS1 cDNA (primer 85S; Table I), confirmed that the PCR product

### Table III

| 5'-Intron boundary (approximate size in kb) | Intron | 3'-Intron boundary |
|--------------------------------------------|--------|-------------------|
| GCCTCACCAG/tgtagtgtgge                  | I (>5) | gctttaccaac/CGATGACTCCGT |
| GCATTCCCCCAGTGG/tgtagtgtggtt           | II (0.3)| /GTCATTCTACTCG |
| TGGTTTTC/ggtaccaat                  | III (3) | tggtttctag/GTCTCACGTTGTTT |
| GACATCAGTG/tgtagcttct                | IV (0.8) | tagtttaacag/GGATATGCT |
| GGAGCTGACAGAAG/tgtagaagagg           | V (2.8) | tgggtagcttct/CITTTCTTCATGCA |
| AAGCTTCAAG/tgtagctttt                | VI (4) | attttctctct/ACATATCA |
| TCATATGG/tgtagatagtaata               | VII (0.9) | eattctctctct/CIATTCA |
| CAGTCGAG/tgtaagggt                    | VIII (2.6) | aattctttatag/GCTTCCACCA |
| GTTGGTGG/tgtagattttg                 | IX (5.2) | ttctttactcttac/GTCTATTGTTTC |
| CTTGGTGT/tgtaagttgctt                | X (4) | aattctttatag/GCTTCCACCA |
| GGGAAAAG/tgtagaggaag                 | XI (1.8) | gtctttactcttac/GCCTATTCA |
| AGGCTCAGCAAG/                         | XII (1.6) | gctttactcttac/GTCTTGA |

Exon-intron boundaries of the 129/J mouse PSS1 gene.
sites (GC boxes) were located at positions 1264 and 1310. Sp1-binding sites have been shown to be widespread in the natural flanking region, 14 bp upstream of the site at which the poly(A) tail is attached to cDNA—3'-untranslated region of the PSS1 gene—3'-RACE PCR using adapter primer Ad68 (see "Experimental Procedures") and primer 620S (Table I) was performed to obtain the sequence of the 3'-untranslated region of the PSS1 cDNA (Fig. 4A). This procedure yielded a 1820-bp product, the partial sequence of which is shown in Fig. 4B. The 3'-untranslated region, from the stop codon (TGA) to the site of polyadenylation (ATTTAA), consists of 929 bp (Fig. 4B). An unusual polyadenylation signal sequence (ATTAAA) is located 14 bp upstream of the site at which the poly(A) tail is attached (40). As depicted in Fig. 4C, the 3'-untranslated region is directly attached to exon 13 of the PSS1 gene, without an intervening intron. The final exon, exon 13, contains 1035 bp and is the largest in the PSS1 gene. Clone 595 F12 lacks 437 bp at the transcriptional initiation site at position 361, and an AML-1a (acute myeloid leukemia factor-1a) site was at position −358. A heat shock factor-2 site was found at position −361, which is the most common transcription-initiating sequence in the murine PSS1 gene. The transcriptional initiation site is boxed and underlined. The consensus sequences of transcription factor-binding elements are underlined. Sp-1, stimulating factor protein-1; USF, upstream stimulatory factor; HSF-2, heat shock factor-2; AML-1a, acute myeloid leukemia factor-1a.

Analysis of the Putative Proximal Promoter Region—Clone 1 (Fig. 1B), which consists of a 5.2-kb BamHI digestion fragment from the PSS1 gene, was identified by Southern blotting using the exon 1-specific primer 88S (Table I) as a probe. Clone 1 contains −4300 bp of the 5'-natural flanking region of the PSS1 gene, all of exon 1 (264 bp), and 700 bp of intron 1. We sequenced 800 bp of the DNA upstream of the transcriptional initiation site, shown as position +1 in Fig. 3, using primers 1U, 2U, 3U, and 4U (Table I). This region of the PSS1 gene did not contain either TATA or CAAT boxes, but did have a high GC content (Fig. 3) (36, 37); 75% of the nucleotides in the first 600 bp were G or C.

Using computer analysis of the 5'-natural flanking region, a number of potential transcription factor-binding sites were revealed, as indicated in Fig. 3. A total of three Sp1-binding sites (GC boxes) were located at positions −12, −70, and −310. Sp1-binding sites have been shown to be widespread in promoters of cellular and viral genes (38) and are usually located close to the transcriptional start site. The promoter of the murine CTP:phosphocholine cytidylyltransferase-a gene, which encodes the rate-limiting enzyme of the CDP-choline pathway for PtdCho synthesis, also contains Sp1-binding sites (39). In the murine PSS1 gene, the Sp1 site at position −310 overlaps with the upstream stimulatory factor site and the N-Myc site. Two GATA-1 and two GATA-2 sites were also identified. One GATA-1 site and one GATA-2 site overlap at position −375. A heat shock factor-2 site was found at position −361, and an AML-1a (acute myeloid leukemia factor-1a) site was at position −548.

was derived from the PSS1 gene (Fig. 2B, lower). To identify the transcriptional initiation site, the 420-bp PCR product was subcloned into the pCR2.1 vector and sequenced in both directions using primers corresponding to the pCR2.1 vector. As shown in Fig. 2C, the transcriptional start site was identified 25 bp upstream of the start of exon 1 and 111 bp upstream of the translational start codon, ATG. The 5'-RACE PCR was performed three additional times using different preparations of total RNA from 129/J mouse liver, with the same result. Transcription begins with an adenine residue, which is the most common transcription-initiating nucleotide.

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**Fig. 2.** Determination of the transcriptional initiation site of the murine PSS1 gene. A, strategy of 5′-RACE PCR using 129J murine liver RNA. The sequences of primers 396AS and 913AS are shown in Table I, and that of primer Race dT is shown under "Experimental Procedures." B, 1.5% agarose gel (upper) and Southern blot analysis of the PCR product using 32P-labeled primer 85S (Table I) (lower) Lanes 1 and 2 contain 50 ng of template; lane 3 contains 100 ng of template. C, deduced sequence of the 5′-end of the PSS1 gene showing the transcriptional initiation site at −25 bp, the start of the first exon at +1 bp, and the ATG translational start site at +86 bp (boldface).
3'-end of the cDNA. However, this portion of the gene does not appear to be interrupted by any introns because (i) the PCR product obtained using genomic DNA as a template and primers specific for these 437 bp is exactly the same size as the PCR product obtained when the cDNA was used as a template; and (ii) according to the sequence obtained from the NCBI Database (accession number AC068091; 966284529-3551-12509), the human PSS1 gene does not contain any introns within the 437 bp at the 3' end of the cDNA.

Tissue Distribution of Murine PSS1 and PSS2—Although several tissues (e.g. brain (11) and liver (23, 24)) have been shown to possess PSS activity, it is unclear what proportion of the total PSS activity in tissues is contributed by PSS1 and PSS2. Our previous Northern blot experiments using a commercially supplied mRNA blot of several murine tissues suggested that PSS1 and PSS2 are expressed to different extents in different tissues (31). We therefore analyzed more carefully the tissue distribution of PSS1 and PSS2 mRNAs using RT-PCR. In addition, we measured the serine, ethanolamine, and choline exchange activities in several tissues. These studies allowed us to determine whether or not a correlation existed between PSS1 and PSS2 mRNA expression and enzymatic activity.

For analysis of mRNA levels, we performed RT-PCR using mRNAs isolated from several tissues of 129/J mice. To avoid cross-reactivity between PSS1 and PSS2 primers, we performed the RT-PCR with primers specific for PSS1 and PSS2 (Fig. 5A) and the internal control cyclophilin. The resulting cDNA was used as a template for PCR using three different primer pairs, one specific for PSS1, one specific for PSS2, and one specific for the internal control cyclophilin. The resulting PCR products were 313 bp for PSS1, 500 bp for PSS2, and 200 bp for cyclophilin. B, the products were analyzed by agarose gel electrophoresis (left panels) and Southern blot analysis (right panels) using oligonucleotide probes 316AS for PSS1 (upper panels), 3S for PSS2 (center panels), and cyc rev2 (primer specific for internal sequence of cyclophilin) (lower panels). PCR amplifications from individual tissues were performed with the same amounts (50–100 ng) of template cDNA. Data are representative of three independent experiments with the same results. Lane 1, adipose; lane 2, brain; lane 3, heart; lane 4, kidney; lane 5, lung; lane 6, liver; lane 7, spleen; lane 8, testis.
examined for enzymatic activities of serine, choline, and ethanolamine exchange (Fig. 6). In *in vitro* enzymatic assays, PSS1 uses choline, ethanolamine, and serine for base exchange, whereas PSS2 uses ethanolamine and serine, but not choline. Choline exchange activity is therefore a marker for the presence of PSS1, whereas ethanolamine and serine exchange activities are contributed by both PSS1 and PSS2. The highest specific activity for serine exchange was in testis and kidney (Fig. 6), corresponding to the high level of expression of PSS1 and PSS2 mRNAs in these tissues (Fig. 5B). Serine exchange activity was also detected in all other tissues examined (brain, liver, lung, skeletal muscle, spleen, and heart). These observations correlate well with the results from the RT-PCR experiments shown in Fig. 5B. The tissue distribution of ethanolamine exchange activity essentially paralleled that of serine exchange activity, with testis and kidney exhibiting the highest ethanolamine exchange activity (Fig. 6), in agreement with the high level of both PSS1 and PSS2 mRNAs detected in these tissues (Fig. 5B). The ethanolamine exchange activity in skeletal muscle, liver, lung, spleen, brain, and heart was 50–70% lower than that in testis and kidney. The highest specific activity for choline exchange, presumably imparted by PSS1, was in brain (Fig. 6). This tissue also showed one of the highest levels of expression of PSS1 mRNA, according to the RT-PCR analysis depicted in Fig. 5B. The choline exchange activity in the eight tissues examined varied over a 5-fold range, with lung having the lowest specific activity and brain, kidney, and liver having relatively high activity (Fig. 6). These activities are in good agreement with the highest level of expression of PSS1 mRNA being in kidney, brain, liver, and heart (Fig. 5B) (31).

The differential tissue expression of PSS1 and PSS2 mRNAs suggests that these two enzymes might have tissue-specific functions.

**Chromosome Localization**—Clone 595 F12 was used as a probe to determine the chromosome localization of the murine PSS1 gene by the FISH mapping technique (41). Under the conditions used, the detection efficiency was 98% for this probe. Among 100 checked mitotic figures, 98 showed hybridization signals on one pair of chromosomes. DAPI banding was also performed to identify the chromosome on which the PSS1 gene was located. The assignment of signals to mouse chromosome 13 was deduced by examining the overlap between the signal from the PSS1 probe and the DAPI signal. The detailed position of the gene on chromosome 13 was further determined to be in region B-C1 from a combination of 10 photographs (Fig. 7B). Other genes identified on murine chromosome 13 are cytidine-monophospho-N-acetylneuraminic acid hydrodrolase, coagulation factor II receptor, 3-hydroxy-3-methylglutaryl-CoA reductase, and phosphatidylinositol 3-kinase regulator-1. The human PSS1 and PSS2 genes are localized to chromosomes 8 and 11, respectively (NCBI Database accession number AC068091; RID 966284529-3551-12509).

**Conclusion**—We have isolated and characterized the murine PSS1 gene and compared its tissue expression with that of the PSS2 gene. The PSS1 gene was localized to mouse chromosome 13 in region B-C1, is ~35 kb in length, and consists of 13 exons and 12 introns. PSS1 and PSS2 mRNAs were widely distributed throughout the murine tissues examined. According to RT-PCR analysis, PSS2 mRNA was most highly expressed in testis, in agreement with the specific activities of serine and ethanolamine exchange also being highest in this tissue. Choline exchange activity, contributed by PSS1, was highest in brain and kidney. In experiments presently underway, we are using this information on the PSS1 gene to generate PSS1 gene-disrupted mice with a goal of determining why mammals have (at least) two genes encoding enzymes that catalyze Ptd-

![Fig. 6. Serine, ethanolamine, and choline exchange activities of murine tissues.](image)

![Fig. 7. Chromosome localization of the murine PSS1 gene. A, diagrammatic representation of the FISH mapping results using the PSS1 BAC clone 595 F12 as a probe.](image)
Ser synthesis.

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