The Selenium-rich C-terminal Domain of Mouse Selenoprotein P Is Necessary for the Supply of Selenium to Brain and Testis but Not for the Maintenance of Whole Body Selenium*

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Selenoprotein P (Sepp1) has two domains with respect to selenium content: the N-terminal, selenium-poor domain and the C-terminal, selenium-rich domain. To assess domain function, mice with deletion of the C-terminal domain have been produced and compared with Sepp1−/− and Sepp1+/+ mice. All mice studied were males fed a semipurified diet with defined selenium content. The Sepp1 protein in the plasma of mice with the C-terminal domain deleted was determined by mass spectrometry to terminate after serine 239 and thus was designated Sepp1240–361. Plasma Sepp1 and selenium concentrations as well as glutathione peroxidase activity were determined in the three types of mice. Glutathione peroxidase and Sepp1240–361 accounted for over 90% of the selenium in the plasma of Sepp1240–361 mice. Calculations using results from Sepp1+/+ mice revealed that Sepp1, with a potential for containing 10 selenocysteine residues, contained an average of 5 selenium atoms per molecule, indicating that shortened and/or selenium-depleted forms of the protein were present in these wild-type mice. Sepp1240–361 mice had low brain and testis selenium concentrations that were similar to those in Sepp1−/− mice but they better maintained their whole body selenium. Sepp1240–361 mice had depressed fertility, even when they were fed a high selenium diet, and their spermatozoa were defective and morphologically indistinguishable from those of selenium-deficient mice. Neurological dysfunction and death occurred when Sepp1240–364 mice were fed selenium-deficient diet. These phenotypes were similar to those of Sepp1−/− mice but had later onset or were less severe. The results of this study demonstrate that the C terminus of Sepp1 is critical for the maintenance of selenium in brain and testis but not for the maintenance of whole body selenium.

Selenoprotein P (Sepp1)3 contains most of the selenium in plasma (1). Rat Sepp1 cDNA codes for 366 amino acid residues, 10 of which are selenocysteines and 17 cysteines (2). Two domains with respect to selenium content are discernable. The N-terminal 244 residues include 1 selenocysteine and 7 cysteines. Two potential redox motifs:40UXC43 and 153CX4156 are present in this domain, allowing the prediction that it has enzymatic properties (3). The smaller C-terminal domain comprises 122 amino acid residues: 9 of them are selenocysteines and 10 are cysteines. Thus, the C-terminal one-third of the protein contains 90% of its selenium, raising the possibility that this domain plays a role in selenium transport. Each domain, then, has been postulated to have a distinct function.

Sepp1 was purified from rat plasma using a monoclonal antibody to the N-terminal domain. After applying the purified protein to a heparin column, we eluted 4 isoforms using a pH gradient (4). Mass spectrometry analysis identified the isoforms as sharing the same amino acid sequence but terminating at positions corresponding to UGAs (which code for selenocysteines) in the open reading frame (5). In addition to the full-length protein, isoforms terminating at the second, third, and seventh in-frame UGAs were identified. Others have studied Sepp1 synthesis in cell culture and have even identified a form with termination at the first in-frame UGA (6). Production of the isoforms found in plasma might occur by termination of translation at UGAs that, if read through, would dictate insertion of selenocysteine or by removal of C-terminal fragments of the full-length protein. The latter possibility might constitute a mechanism of selenium delivery. In any case, the discovery of the isoforms supported the postulates that the two domains had distinct functions.

Mouse Sepp1 has 84% sequence identity with the rat protein and the same apparent domains. Mice with the Sepp1 gene (Sepp1) deleted have been produced and found to have depressed whole body selenium caused by excessive selenium excretion in the urine (7). In addition, they have sharply

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§ This abbreviation is used: Sepp1, selenoprotein P protein; Sepp1, selenoprotein P protein; Sepp1240–361, truncated selenoprotein P protein; Sepp1240–364, truncated selenoprotein P gene; PBS, phosphate-buffered saline; LC-MS, liquid chromatography-mass spectrometry; MS, mass spectrometry; ELISA, enzyme-linked immunosorbent assay; UTR, untranslated region.
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depressed testis and brain selenium concentrations and dysfunction of those two organs (8). Feeding a high selenium diet prevented brain dysfunction (9), but abnormal spermatozoa were produced regardless of the dietary selenium content (10). When hepatic Sepp1 synthesis was suppressed by hepatic specific deletion of the tRNA for selenocysteine, a sharp decrease in kidney selenium occurred, leading to the hypothesis that Sepp1 transports selenium to the kidney in a specific manner (11). All these findings support the hypothesis that Sepp1 is involved in selenium transport and metabolism in the mouse.

As a strategy to investigate the two domains of Sepp1 in vivo, mice with deletion of the C-terminal domain have been produced. We have named the resulting truncated protein Sepp1Δ240−361 to reflect the deletion of amino acid residues 240−361. These residues correspond to residues 245−366 in the rat protein and include 9 of 10 selenocysteines. The present report describes the production and characterization of these mice.

EXPERIMENTAL PROCEDURES

Reagents—Restriction enzymes and ligases were purchased from Promega (Madison, WI), New England Biolabs (Beverly, MA), and MBI Fermentas (Amherst, NY). Cloning vectors, pBluescript and pBC, were purchased from Stratagene (La Jolla, CA). The loxP flanked neo gene, pKT1loxA, (12), the TK2 gene (13), and the TK1−TK2 cassette were generous gifts of Dr. Kirk R. Thomas, University of Utah. Oligonucleotides used as primers for library screening, vector construction, and PCR amplification reactions were synthesized by core laboratory facilities at Vanderbilt University Medical Center and the University of Utah. [32P]dATP and [32P]dCTP were purchased from PerkinElmer Life Sciences. [75Se]selenite (specific activity: 1000 mCi/mg selenium) was purchased from the University of Missouri Research Reactor Facility, Columbia, MO. NADPH and [35S]selenite (specific activity: 1000 mCi/mg selenium) were supplied by the Mouse Core Facility at the University of Utah using published procedures (14, 15). PCR assays were used to determine the genotype of ES cells and adult mice as described previously (8). The primers used to screen for ES-positive cells were MoSePA11 (5′-CCACTCTGTAATCTGACGGCT-3′) and ACNeoS1 (5′-CCTTCTATCGCCCTTGA-3′). Cells containing the mutant gene gave a PCR product of 2.7 kb. The primers used to screen tissue DNA were MoSePS18 (5′-CGAGGAGCATCCTTTGGCAGCT-3′) and MoSePA19 (5′-CAGTGGAGTACCTTTGGAC-3′). Cells containing the mutant gene gave a PCR product of 3.7 kb. The primers used to screen tissue DNA were MoSePS18 and MoSePA19. The primers used to screen tissue DNA were MoSePS18 and MoSePA19. The primers used to screen tissue DNA were MoSePS18 and MoSePA19. The primers used to screen tissue DNA were MoSePS18 and MoSePA19. The primers used to screen tissue DNA were MoSePS18 and MoSePA19.
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with aspen shavings as bedding material. The light:dark cycle was 10 h:14 h. Breeding mice received rodent chow (Lab Diet 5001, Richmond, IN) while nursing dams received rodent maternity chow (Lab Diet 5015). Male mice were used for experiments and they received pelleted Torula yeast-based diet supplemented with selenium as required for each study. The basal form of the Torula-yeast diet contained <0.02 mg selenium/kg (8). Sodium selenite was added to this diet during mixing to give the desired selenium content. The diet was mixed and pelleted to our specifications by Harlan-Teklad (Madison, WI). All mice received food and water ad libitum. The Vanderbilt University Institutional Animal Care and Use Committee approved animal protocols for studies at Vanderbilt and the corresponding University of Utah committee approved the protocols used to generate the mutant mouse.

In experiments that required tissues to be harvested, mice were anesthetized with isoflurane and exsanguinated by removal of blood from the inferior vena cava. Blood was treated with Na2EDTA (1 mg/ml) to prevent coagulation and was separated by centrifugation. Liver, kidney, testis, and brain of pregnancies and progeny were recorded. If there was no evidence of a pregnancy by day 21, the female was exposed to a different male for a 4-day period. Each male was thus exposed to 6 different females, and the numbers of pregnancies and progyny were recorded.

Fertility Studies—Sepp1Δ240–361 and C57BL/6 male mice (2–3 months of age) were exposed to C57BL/6 female mice for periods of 4 days. The female mice were examined for evidence of pregnancy beginning at day 14 after initial exposure to a male. If there was no evidence of a pregnancy by day 21, the female was exposed to a different male for a 4-day period. Each male was thus exposed to 6 different females, and the numbers of pregnancies and progyny were recorded.

Analysis of Spermatozoa from Sepp1Δ240–361—The caput and cauda regions of the epididymis were minced in Dulbecco’s PBS. The sperm suspension was fixed by adding an equal volume of 4% formaldehyde buffered with 0.1 M sodium phosphate, pH 7.4, and spermatozoa were examined by phase contrast microscopy.

Biochemical Measurements—Plasma glutathione peroxidase activity was measured by the coupled method with 0.25 mM hydrogen peroxide as substrate (16). Plasma Sepp1 was measured by ELISA. The monoclonal antibody 954 was used as the capture antibody, and the polyclonal antibody preparation 695, obtained from a rabbit immunized with rat Sepp1, was used as the detection antibody. Antibody preparation 695 had been immunoaffinity-purified from serum using a mouse Sepp1 column. The ELISA protocol required coating wells of a microtiter plate with 954 (0.1 μg/well) followed by blocking with Block Ace (Serotec, Ltd, Raleigh, NC). After washing, the plate was incubated with sample (50 μl of preprepared plasma) and purified antibody preparation 695 (50 μl of 1 μg/ml) at 37°C for 30 min. Horseradish peroxidase-conjugated goat anti-rabbit IgG (whole molecule) was used to detect the amount of 695 bound to Sepp1 or Sepp1Δ240–361 captured by 954. TMB Microwell Peroxidase Substrate System (KPL, Gaithersburg, MD) was used for color development with 1 N H2SO4 as the stop solution. The developed color was measured at 450 nm with a Multiskan Spectrum plate reader (ThermoElectron Corporation, Vantaa, Finland). Plasma samples were prepared prior to assay by dilution in PBST (PBS with 0.05% Tween-20) containing 2% rat plasma cleared of Sepp1 by monoclonal antibody 8F11 (17) immunoaffinity purification. Typically 0.1 μl of normal mouse plasma was used in the ELISA assay. Larger amounts were required for plasma from selenium-deficient mice. Each assay plate contained a standard curve constructed with 954 immunoaffinity-purified mouse Sepp1 (0–7.5 ng of Sepp1). The immunoaffinity column was prepared according to the manufacturer’s directions using 954 monoclonal antibody and Amino- link Coupling Gel (Pierce). Selenium was measured using a modification of the fluorometric assay of Koh and Benson (18, 19). The lower limit of detection of this assay is 1 ng of selenium.

Purification and Mass Spectrometry Studies of Sepp1Δ240–361—Plasma was obtained from Sepp1Δ240–361 mice. Sepp1Δ240–361 was purified using a 954 immunoaffinity column. Purified protein was subjected to SDS-PAGE on a 10% Ready Gel (Bio-Rad). After staining with Coomassie Blue, the protein band was excised. After 1 h of in-gel digestion with Trypsin Gold (Promega Corp., Madison, WI), the peptides were identified by LC-MS.

The LC-MS/MS analyses were performed on a ThermoFinnigan LTQ linear ion trap mass spectrometer equipped with a ThermoFinnigan Surveyor LC pump and autosampler, NanoSpray source (Thermo Electron), and Xcalibur 1.4 instrument control and data analysis software. HPLC separation of the tryptic peptides was achieved with 100 mm × 11 cm C-18 capillary column (Monitor C18, 5 micron, 100 Å, Column Engineering), at 0.7 l min−1 flow rate. Solvent A was H2O containing 0.1% formic acid and solvent B was acetonitrile containing 0.1% formic acid. The gradient program was: 0–3 min, linear gradient from 0 to 5% B; 3–5 min, 5% B; 5–50 min, linear gradient to 50% B; 50–52 min, linear gradient to 80% B; 52–55 min, linear gradient to 90% B; 55–56 min, 90% B in solvent A. MS/MS scans were acquired using an isolation width of 2 m/z, an activation time of 30 ms, and activation Q of 0.250 and 30% normalized collision energy using 1 microscan and ion time of 100 for each MS/MS scan. The mass spectrometer was tuned prior to analysis using the synthetic peptide TpepK (AVAGKAGAR); some parameters may have varied slightly from experiment to experiment, but typically the tune parameters were as follows: spray voltage of 2.0 kV, a capillary temperature of 160°C, a capillary voltage of 60 V and tube lens 130 V. Initial tandem MS analysis was performed using data-dependent scanning in which one full MS spectrum, using a full mass range 400–2000 amu, was followed by 3 MS/MS spectra. Peptides and modified peptides were identified using the SEQUEST algorithm (20) and the SEQUEST Browser software (Thermo Electron, San Jose, CA) using the mouse subset of the Uniref100 data base and the search was appropriately modified using differential modifications to account for the selenocysteine residues in the sequence. In addition, lists of theoretical or SEQUEST identified peptides were created, and each peptide was run through P-Mod software to check for possible sequence variance or variable cleavage (21). Once the candidate sequence was identified, a targeted analysis was performed in which the peptide mass was selected for MS/MS analysis and fragment ions were selected for MS/MS/MS fragmentation to confirm the identification of the fragment ions.
75Se Labeling of Mice—Sepp1+/
+ and Sepp1Δ240–361 mice were injected intraperitoneally with 10 μCi of [75Se]selenite in 0.15 M NaCl. Blood was obtained from the mice 3.5 h after 75Se administration. Plasma was separated by centrifugation and subjected to SDS-PAGE, along with a lane of molecular weight markers. After staining with Coomassie Blue, the gel was dried and exposed to Kodak XAR film.

Statistics—Results were analyzed using Student’s t test or using analysis of variance with post hoc analysis for statistical differences using Tukey’s Multiple Comparison test. Significance was set at p < 0.05. All calculations, including statistical comparison of survival curves, were performed on a Macintosh G5 using GraphPad Prism Ver 4.0b (GraphPad Software, San Diego, CA).

RESULTS

Generation of Sepp1 Mutant Mice Expressing Sepp1Δ240–361—Mutant mice were produced in which protein synthesis was expected to terminate at the second UGA of Sepp1. This truncation of Sepp1 was accomplished by insertion of a TAA immediately after the second in-frame TGA (Fig. 1). The deletion was intended to result in a truncated plasma protein containing 239 amino acid residues, only one of which was a selenocysteine.

For construction of the deletion mutant targeting vector, a 13-kb SpeI fragment of a mouse genomic P1 DNA clone was used (8). A Neo cassette capable of self-excision when passing through the male germ line of mice was inserted at a BglII site engineered into exon 5, just after the second TGA of the Sepp1 gene. In between this TGA and the BglII site, a true stop codon (TAA) was also introduced to ensure the termination of translation.

Presence of Truncated Sepp1 in Plasma and Verification of Its Translation Termination Site—Plasma was collected from Sepp1+/+ and Sepp1Δ240–361 mice that had been injected with a tracer dose of [75Se]selenite. The plasma was subjected to SDS-PAGE and autoradiography (Fig. 2A). The 75Se band in the Sepp1+/+ mouse plasma migrated at 49,000 Da (lane 1), and there was no corresponding band in Sepp1Δ240–361 mouse plasma (lane 3). Rather, a somewhat less intense band migrated at 41,200 Da in lane 3. The predicted peptide masses of Sepp1 and Sepp1Δ240–361 are 40,692 Da and 27,053 Da, respectively.
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A.  

B.  

C.  

The N-terminal domain of rat Sepp1 is heavily glycosylated (22), and the presence of carbohydrate might contribute to the reduced migration of the mouse proteins, leading to an increase in the weight estimates by SDS-PAGE. Excess Sepp1\(^{+/+}\) plasma was applied to lane 2. \(^{75}\)Se is seen to extend downward from the main band in this lane, indicating the presence of \(^{75}\)Se-containing molecules that were smaller than the major one(s).

Immunoaffinity purification of Sepp1 from plasma of Sepp1\(^{\Delta240-361}\) mice was carried out. Mass spectrometry of trypsin digests of the purified protein yielded a coverage map with the C-terminal amino acid being serine at position 239 (Fig. 2B). The analysis of that peptide at the C terminus of Sepp1\(^{\Delta240-361}\) is shown in Fig. 2C. These results verify that the mutant mice have a form of Sepp1 in their plasma that is truncated after the serine residue at position 239.

Plasma Selenium Biomarkers in Sepp1\(^{\Delta240-361}\) Mice—The two plasma selenoproteins, Sepp1 and glutathione peroxidase, are often used as selenium biomarkers (23). These biomarkers were compared in the three groups of mice with different Sepp1 status: Sepp1\(^{\Delta240-361}\) mice, Sepp1\(^{+/+}\) mice, and Sepp1\(^{-/-}\) mice (Table 1). All mice were selenium replete, having been fed a diet supplemented with 1.0 mg of selenium/kg. Glutathione peroxidase was the only selenium biomarker in the plasma of the Sepp1\(^{-/-}\) mice, and its activity in them corresponded to a plasma selenium concentration of 66 \(\mu\)g of selenium/liter (Table 1). Extrapolating to the other groups on the basis of glutathione peroxidase activity, plasma from the Sepp1\(^{\Delta240-361}\) mice would contain 80 \(\mu\)g of selenium/liter and plasma from the Sepp1\(^{+/+}\) mice would contain 77 \(\mu\)g of selenium/liter in the form of glutathione peroxidase.

The Sepp1 concentration value for Sepp1\(^{\Delta240-361}\) mouse plasma was higher than the one for Sepp1\(^{+/+}\) mouse plasma. The immunoassay recognizes predominantly the N terminus of the protein, and thus the results are essentially a molar comparison of Sepp1 and Sepp1\(^{\Delta240-361}\). Based on the Sepp1-predicted peptide mass of 40,692 Da, the Sepp1\(^{+/+}\) mice had 0.71 \(\mu\)mol/liter and the Sepp1\(^{\Delta240-361}\) mice had

**FIGURE 2.** A, autoradiograph of SDS-PAGE gel of plasma from a Sepp1\(^{+/+}\) mouse (lanes 1 and 2) and a Sepp1\(^{\Delta240-361}\) mouse (lane 3). The mice were fed a diet containing 1.0 mg selenium/kg. Each mouse was injected with 10 \(\mu\)Ci \(^{75}\)Se as selenite 3.5 h before plasma was sampled. Lanes 1 and 3 had 0.5 \(\mu\)l of plasma applied, and lane 2 had 3 \(\mu\)l applied. B, and C, identification of the C terminus of the mutant form of Sepp1. B shows a coverage map of tryptic peptides of the protein purified from plasma of Sepp1\(^{\Delta240-361}\) mice (underlined residues). U represents selenocysteine in the amino acid sequence of Sepp1. The most C-terminal peptide detected is shown shaded and in a box. C shows an MS/MS spectrum of doubly charged peptide with \(m/z\) of 574.7 (the most C-terminal peptide of the coverage map in B). Predominance of doubly charged b- and y-ions is consistent with presence of basic residues, and these ions were confirmed by MS/MS analysis (data not shown).

**TABLE 1**

| Mice  | \(n\) | Seleniuma | Sepp1b | Glutathione peroxidaseb |
|-------|------|-----------|--------|------------------------|
|       | \(\mu\)g/liter | mg/liter | units/liter |
| Sepp1\(^{+/+}\) | 6 | 375 ± 16c | 29 ± 4d | 348 ± 61   |
| Sepp1\(^{\Delta240-361}\) | 6 | 178 ± 11c | 42 ± 3  | 359 ± 104 |
| Sepp1\(^{-/-}\) | 9 | 66 ± 14c | Not detectable | 298 ± 74    |

* A mice were fed a diet supplemented with 1 mg of selenium/kg as selenite for 3–7 months from weaning.

b Values are means ± S.D.

c,d Values with the same superscript are different from one another, \(p < 0.001\).
1.03 μmol/liter. In the Sepp1Δ240−361 mice, the protein had one selenium atom per molecule so it should have contributed 82 μg of selenium/liter plasma. When this value is added to the 80 μg of selenium/liter calculated to be in glutathione peroxidase, a total plasma selenium concentration of 162 μg/liter is estimated to be accounted for by the two selenoproteins. This compares favorably with the determined selenium concentration of 178 μg/liter (Table 1) and supports the validity of using this assay to determine the molar concentration of Sepp1Δ240−361.

If Sepp1 in the Sepp1+/+ mice had contained the full 10 selenium atoms per molecule, it would have contributed 560 μg of selenium/liter plasma. When the 77 μg of selenium/liter calculated to be in glutathione peroxidase is subtracted from the 375 μg/liter that was determined, only 298 μg of selenium/liter are available for the selenium content of Sepp1. Thus, some of the Sepp1 molecules in Sepp1+/+ mouse plasma must contain fewer than 10 selenium atoms. Based on the above calculations, Sepp1 contains an average of 5 selenium atoms per molecule in Sepp1Δ+/+ mice. Thus, forms of mouse Sepp1 containing reduced numbers of selenium atoms are present in the plasma of Sepp1Δ+/+ mice.

**TABLE 2**

| Sepp1Δ240−361 | Sepp1Δ−/− | p |
|---------------|-----------|---|
| ng/g body weight | 202 ± 34 (5) | 240 ± 18 (4) | 0.09 |
| − | 162 ± 10 (5) | 255 ± 34 (5) | 0.0004 |

* Values are means ± S.D. (n).

Whole Body and Tissue Selenium Concentrations in Sepp1Δ240−361 Mice—Whole body selenium was measured in Sepp1Δ240−361 and Sepp1Δ−/− mice fed a diet supplemented with 0.25 mg/kg to compare the effect of the loss of the C-terminal domain with the effect of the loss of the complete protein. Table 2 shows whole body selenium concentrations of those mice compared with litter-matched Sepp1Δ+/+ controls. Whole body selenium concentration appeared to decline in both groups, but the apparent decrease did not reach statistical significance in the Sepp1Δ240−361 mice. Whole body selenium in the Sepp1Δ−/− mice was 36% below that in Sepp1Δ+/+ controls, and this difference was highly significant, consistent with a previous report (7). Thus, whole body selenium is highly affected by deletion of Sepp1 but not by loss only of its C terminus.

Fig. 3 shows that deletion of the C terminus had large effects on brain and testis selenium, regardless of the amount of selenium fed. Kidney selenium was not affected by loss of the C terminus when the mice were fed the nutritional requirement of selenium (0.1 mg/kg diet), but a modest effect on kidney selenium was present at higher dietary selenium levels. These results suggest that whole body and kidney selenium depend more on the N terminus of Sepp1 than on its C terminus, while the brain and testis depend more on the C terminus.

**Effect of Sepp1Δ240−361 on Male Reproduction**—Deletion of the C-terminal domain of Sepp1 had a sharp lowering effect on testis selenium levels (Fig. 3) and resulted in production of spermatozoa with defects (Fig. 4) similar to those seen in selenium-deficient mice and in Sepp1Δ−/− mice (10). Spermatozoa from the caput epididymis of Sepp1Δ240−361 males possessed a flagellar defect that appeared identical to that previously identified in spermatozoa of both selenium-deficient Sepp1Δ+/+ mice and selenium-replete Sepp1Δ−/− mice (10). The flagellum displayed an abrupt narrowing of the posterior midpiece (Fig. 4A), which was shown previously to reflect a premature truncation of the mitochondrial sheath (10). Further alterations of sperm flagellar structure were apparent in spermatozoa from the cauda region of the epididymis. Most spermatozoa displayed a hairpin bend at the junction of the midpiece and principal piece (Fig. 4B). However, some spermatozoa were detected that had an extended flagellum. Most of these displayed narrowing of the flagellum immediately proximal to the midpiece–principal piece junction (Fig. 4B).

To assess the fertility of Sepp1Δ240−361 males, they were mated with Sepp1Δ+/+ females (Table 3). These matings resulted in 31% as many pregnancies as occurred when Sepp1Δ+/+ males were mated with Sepp1Δ+/+ females. The number of live progeny per litter was 63% of the number of live progeny from matings of Sepp1Δ+/+ males and females. Only one pup (5%) survived to weaning from the Sepp1Δ240−361 sired litters compared with 49% of the pups from Sepp1Δ+/+ sired litters. The pregnancy rate in this experiment was greater than the rate we reported previously with Sepp1Δ−/− sires (8, 10), but it was still depressed compared with Sepp1Δ+/+ sires. Also, survival from birth to weaning was lower in pups sired by Sepp1Δ240−361 males, showing an effect on the fitness of pups after birth. These results indicate that the effects of Sepp1 on male reproduction...
are largely caused by the C-terminal domain but leave open the possibility that the N-terminal domain has some effect.

**Effect of Selenium Deficiency on Neurological Function and Survival of the Sepp1Δ240–361 Mice**—Feeding selenium-deficient diet to Sepp1−/− mice resulted in neurological dysfunction and death (8, 9). Similar, but not identical, results were obtained when Sepp1Δ240–361 mice were fed selenium-deficient diet. While Sepp1−/− mice fed selenium-deficient diet from weaning demonstrated a steady progression of neurological signs (see Table 2 in Ref. 9), Sepp1Δ240–361 mice did not. Fewer clinical abnormalities were noted prior to death of the selenium-deficient Sepp1Δ240–361 mice (15 mice observed). One mouse was found dead at 14 days. The first sign in the other 14 Sepp1Δ240–361 mice, occurring 2–3 weeks after weaning, was a fine tremor and excitability. This nervous behavior progressed to distinct hyperactive episodes in 12 of the Sepp1Δ240–361 mice, and 6 of those mice were unable to right themselves. Once a hyperactive episode had been observed, death or >20% weight loss, necessitating euthanasia, occurred within 2–3 days.

Survival curves for Sepp1Δ240–361 and Sepp1−/− mice fed selenium-deficient diet from weaning show that deletion of the entire protein was lethal in a shorter time than deletion of only the C-terminal domain (Fig. 5). Decreased stride length was the first indication of neurological dysfunction in Sepp1−/− mice (9). Selenium supplementation of the diet did not completely correct the shortened stride. Stride length was compared in Sepp1Δ240–361 and Sepp1−/− mice fed diets supplemented with 0.1–0.5 mg selenium/kg (Fig. 6). The shortened stride length in the Sepp1−/− mice was observed at all dietary levels of selenium supplementation, while stride lengths of Sepp1Δ240–361 mice were not different from those of Sepp1−/− mice at any level of selenium supplementation.

These results indicate that the C-terminal domain of Sepp1 is responsible for most of the effects of the protein on the nervous system. However, subtle differences between Sepp1Δ240–361 mice and Sepp1−/− mice point to roles for the N-terminal domain as well.

**DISCUSSION**

The absence from plasma of 75Se migrating at 49,000 Da (Fig. 2A, lane 3) and the presence of a shortened version of Sepp1 with serine 239 at its C terminus (Fig. 2) demonstrate that replacement of Sepp1 with Sepp1Δ240–361 has been achieved. Mass spectrometry characterization of the protein was necessary to determine whether a second selenocysteine was present as residue 240. It was not. The TGA corresponding to that position was the last codon before the TAA in the vector that was used to produce Sepp1Δ240–361 mice. The fact that the UGA at that position was not translated as a selenocysteine residue cannot be attributed to altered spacing of the SECIS elements
because that spacing was essentially unchanged in the vector. Altered 3′ context has been shown to affect UGA readthrough (24, 25), and it appears to be the most likely cause for this UGA terminating translation instead of specifying insertion of selenocysteine.

Molar concentrations of Sepp1Δ240–361 in plasma were higher than those of Sepp1 in wild-type mice (Table 1), suggesting that folding, quality control, and secretion of the shortened form took place efficiently. Thus, we predict that the Sepp1Δ240–361 mice will have preserved function of the N-terminal domain of Sepp1 but no function of the C-terminal domain. They should, therefore, be suitable for studies to distinguish the functions of the two domains.

Deletion of Sepp1 is associated with several phenotypes in mice. Comparing those phenotypes with the phenotypes of Sepp1Δ240–361 mice provides insight into the functions of the domains of Sepp1. The decrease in whole body selenium in Sepp1−/− mice, shown in them to be caused by increased excretion of selenium in the urine (7), was not present in Sepp1Δ240–361 mice (Table 2). We postulated that hepatic production of the urinary excretory metabolites of selenium is in competition for metabolically available selenium with Sepp1 production and secretion (7). Elimination of Sepp1 would therefore allow more of the available selenium to enter the pathway leading to excretory metabolites, lowering whole body selenium. Elimination of the selenium-rich C-terminal domain would be expected to decrease the amount of selenium used in synthesis of the Sepp1 protein (Sepp1Δ240–361 in this case) and thereby also decreases whole body selenium. We did not observe a statistically significant decrease in Sepp1Δ240–361 mice, however (Table 2).

This result suggests that if the hypothesis we put forward is correct, enough selenium was consumed in the synthesis of Sepp1Δ240–361 to compete with urinary metabolite production and prevent urinary wasting to the extent that occurs in Sepp1−/− mice.

Selenium in brain and testis is highly dependent on Sepp1 (8), and we have postulated that selenium acquisition from Sepp1 is receptor-mediated in these tissues. Others have concluded that kidney selenium is also Sepp1-dependent (11). Fig. 3 shows that Sepp1Δ240–361 mice have very sharp decreases in brain and testis selenium concentrations that are similar to those that occur in Sepp1−/− mice (8), implicating the C-terminal domain of Sepp1 as the major source of their selenium. Kidney selenium, however, was not at all or only slightly decreased in Sepp1Δ240–361 mice compared with Sepp1−/− mice (Fig. 3). This implies that the C-terminal domain is not a major source of kidney selenium. Some potential explanations of this are: 1) that the N terminus supplies selenium to the kidney directly and 2) that kidney selenium mirrors whole body selenium. The latter explanation implies that the N-terminal domain supplies selenium to the kidney indirectly. This seems a less likely explanation, however, because no decrease in kidney selenium was found in Sepp1Δ240–361 mice fed a diet supplemented with 0.1 mg of selenium/kg (Fig. 3), while this condition led to the greatest decrease in kidney selenium in Sepp1−/− mice (8). Therefore, it is more likely that the N-terminal domain supplies selenium directly to the kidney. If, as suggested here, different domains of Sepp1 supply selenium to different tissues, separation of the domains is likely to occur in the animal.

Isoforms of Sepp1 are present in rat plasma (4, 5, 26). The results in Table 1 indicate that mouse plasma Sepp1 contains, on average, 5 selenium atoms per molecule while 10 are predicted to be in the full-length molecule. Thus, shortened and/or selenium-depleted forms of Sepp1 are present in the plasma of Sepp1+/− mice. Evidence of shortened forms can be seen in Fig. 2A (lane 2). These shorter forms would contain less 75Se than full-length Sepp1 and thus require application of greater amounts of plasma for their detection. The nature of these forms of Sepp1, presumably containing fewer than 10 selenium atoms, has not been determined. They might terminate at in-frame UGAs as we have demonstrated in rats and as others have demonstrated in cultured cells (6).

It is possible that full-length and shortened forms of Sepp1 are secreted in the mouse and that the full-length form provides selenium to the brain and testes while the shortened forms provide it to the kidney. Another possibility is that only the full-length form of Sepp1 is secreted, and a mechanism exists in some tissues to remove a C-terminal portion of it. The resulting N-terminal fragment might provide selenium to the kidney. The first possibility is supported by phylogenetic considerations. Zebrafish, D. rerio, have a full-length Sepp1 gene (it has 17 UGA codons instead of the 10 in its mammalian counterparts). D. rerio also has a second Sepp1 gene that is similar to Sepp1Δ240–361. It has just one selenocysteine codon and when expressed in mammalian cells, its product is secreted (27). Though the present work utilized a deletion for its functional studies, it is directly relevant to a role for the rat Sepp1 isoform we found earlier with a single selenocysteine (5, 26). Recent findings about a differential role for the two SECIS elements in the 3′-UTR of Sepp1 mRNAs and mRNA...
structures adjacent to at least a substantial number of selenocysteine-specifying UGA codons (6, 24) provide a starting point for the important task of discovering how this and the other isoforms are generated.

The clinical phenotypes of Sepp1−/− mice are primarily deficiencies of selenium in testis and brain. Fertility of Sepp1Δ240–361 male mice is reduced (Table 3), but not as severely as fertility of Sepp1−/− male mice (8, 10). Thus, it is possible that Sepp1Δ240–361 is able to transport a small amount of selenium to the testis. Alternatively, the increased whole-body selenium in Sepp1Δ240–361 mice over that of Sepp1−/− mice might increase testis selenium supply. However, it is clear from Fig. 3 that the C-terminal domain of Sepp1 provides most of the testis selenium.

Sepp1, and therefore presumably Sepp1Δ240–361, is expressed by many cells in the brain (29, 30). Therefore, unlike the seminiferous tubules in which Sepp1 is not expressed (28), the brain appears to depend on at least two Sepp1 functions: one is for Sepp1 in plasma to transport selenium to the brain from the liver and other tissues and the other is for Sepp1 synthesized within the brain to preserve brain selenium content. Determining the details of these functions will require additional work.

Brain function is severely compromised in Sepp1Δ240–361 mice fed a selenium-deficient diet but not as severely as in Sepp1−/− mice fed the same diet. Moreover, there are modest qualitative differences in neurological dysfunction observed between the two types of mice. This points to roles for both domains of Sepp1 in the brain, although the C-terminal domain is clearly the major one maintaining the brain selenium content.

In conclusion, mice with the C-terminal domain of Sepp1 deleted have been produced. They have neurological and male reproductive phenotypes similar to those of mice lacking Sepp1 altogether, although the phenotypes are slightly less severe in Sepp1Δ240–361 mice than in Sepp1−/− mice. Additionally, the N-terminal domain allows conservation of whole body selenium and appears to supply selenium to the kidney. The Sepp1Δ240–361 mice will be useful in further studies of Sepp1 function, especially in seeking biochemical functions of the N-terminal domain.

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REFERENCES

1. Burk, R. F., and Hill, K. E. (2005) Annu. Rev. Nutr. 25, 215–235
2. Hill, K. E., Lloyd, R. S., Yang, J.-G., Read, R., and Burk, R. F. (1991) J. Biol. Chem. 266, 10050–10053
3. Saito, Y., Sato, N., Hirashima, M., Takebe, G., Nagasawa, S., and Takahashi, K. (2004) Biochem. J. 381, 841–846
4. Chittum, H. S., Himeno, S., Hill, K. E., and Burk, R. F. (1996) Arch. Biochem. Biophys. 325, 124–128
5. Ma, S., Hill, K. E., Caprioli, R. M., and Burck, R. F. (2002) J. Biol. Chem. 277, 12749–12754
6. Stoytcheva, Z., Tujeabjeva, R. M., Harney, J. W., and Berry, M. J. (2006) Mol. Cell Biol. 26, 9177–9184
7. Burk, R. F., Hill, K. E., Motley, A. K., Austin, L. M., and Norworthy, B. K. (2006) Biochim. Biophys. Acta 1760, 1789–1793
8. Hill, K. E., Zhou, J., McMahan, W. J., Motley, A. K., Atkins, J. F., Gesteland, R. F., and Burk, R. F. (2003) J. Biol. Chem. 278, 13640–13646
9. Hill, K. E., Zhou, J., McMahan, W. J., Motley, A. K., and Burk, R. F. (2004) J. Nutr. 134, 157–161
10. Olson, G. E., Winfrey, V. P., Nagdas, S. K., Hill, K. E., and Burk, R. F. (2005) Biol. Reprod. 73, 201–211
11. Schweizer, U., Streffkuss, F., Pelt, P., Carlson, B. A., Hatfield, D. L., Körhle, J., and Schomburg, L. (2005) Biochem. J. 386, 221–226
12. Greer, J. M., and Caprioli, M. R. (2002) Neuron 33, 23–34
13. Deng, C., Thomas, K. R., and Caprioli, M. R. (1993) Mol. Cell Biol. 13, 2134–2140
14. Thomas, K. R., and Caprioli, M. R. (1987) Cell 51, 503–512
15. Thomas, K. R., and Caprioli, M. R. (1990) Nature 346, 847–850
16. Lawrence, R. A., and Burk, R. F. (1976) Biochem. Biophys. Res. Commun. 71, 952–958
17. Read, R., Bellew, T., Yang, J.-G., Hill, K. E., Palmer, I. S., and Burk, R. F. (1990) J. Biol. Chem. 265, 17899–17905
18. Koh, T. S., and Benson, T. H. (1983) J. Assoc. Off. Anal. Chem. 66, 918–926
19. Sheehan, T. M., and Gao, M. (1990) Clin. Chem. 36, 2124–2126
20. Eng, J. K., McCormack, A. L., and Yates, J. R. (1994) J. Am. Soc. Mass Spectrom. 5, 976–998
21. Hansen, B. T., Davey, S. W., Ham, A.-J., and Liebler, D. C. (2005) J. Proteome Res. 4, 358–368
22. Ma, S., Hill, K. E., Burck, R. F., and Caprioli, R. M. (2003) Biochemistry 42, 9703–9711
23. Xia, Y., Hill, K. E., Byrne, D. W., Xu, J., and Burck, R. F. (2005) Am. J. Clin. Nutr. 81, 829–834
24. Howard, M. T., Aggarwal, G., Anderson, C. B., Khatri, S., Flanigan, K. M., and Atkins, J. F. (2005) EMBO J. 24, 1596–1607
25. McCaughan, K. K., Brown, C. M., Dalphin, M. E., Berry, M. J., and Tate, W. P. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 5431–5435
26. Himeno, S., Chittum, H. S., and Burck, R. F. (1996) J. Biol. Chem. 271, 15769–15775
27. Kryukov, G. V., and Gladyshev, V. N. (2000) Genes Cells 5, 1049–1060
28. Koga, M., Tanaka, H., Yomogida, K., Tsuchida, J., Uchida, K., Kitamura, M., Sakoda, S., Matsumiya, K., Okuyama, A., and Nishimune, Y. (1998) Biol. Reproduction 58, 261–265
29. Saito, Y., Sato, N., Hirashima, M., Takebe, G., Nagasawa, S., and Takahashi, K. (2004) Biochem. J. 381, 841–846
30. Yang, X., Hill, K. E., Maguire, M. J., and Burck, R. F. (2000) Biochim. Biophys. Acta 1474, 390–396

4 Allen Brain Atlas (2005) Allen Institute for Brain Science.