Long-isoform mouse selenoprotein P (Sepp1) supplies rat myoblast L8 cells with selenium via endocytosis mediated by heparin-binding properties and apolipoprotein E receptor-2 (apoER2)*

Suguru Kurokawa¹, Kristina E. Hill¹, W. Hayes McDonald², and Raymond F. Burk¹

Division of Gastroenterology, Hepatology, and Nutrition, Department of Medicine¹ and Vanderbilt Proteomics Laboratory in the Mass Spectrometry Research Center, Department of Biochemistry², Vanderbilt University School of Medicine, Nashville, Tennessee

Running title: Sepp1 supplies selenium to a muscle cell line via apoER2

To whom correspondence should be addressed: Raymond F. Burk, 1030C Medical Research Building IV, Vanderbilt Medical Center, Nashville, TN 37232-0252, phone 615-343-4748, fax 615-343-6229, e-mail raymond.burk@vanderbilt.edu.

Key words: selenoprotein P, apoER2, heparin binding, endocytosis, selenium supply to cells

Background: ApoER2 endocytosis of Sepp1 supplies testis and brain with Se but the mechanism of supply to other tissues is not known.

Results: Sepp1 supplies Se to heart and skeletal muscle cell lines via apoER2 and many tissues express apoER2.

Conclusion: ApoER2 endocytosis of Sepp1 supplies Se to many tissues.

Significance: ApoER2 uptake of Sepp1 likely regulates Se distribution in the whole body.

SUMMARY

In vivo studies have shown that selenium is supplied to testis and brain by apoER2-mediated endocytosis of Sepp1. Although cultured cell lines have been shown to utilize selenium from Sepp1 added to the medium, the mechanism of uptake and utilization has not been characterized. Rat L8 myoblast cells were studied. They took up mouse Sepp1 from the medium and used its selenium to increase their glutathione peroxidase (Gpx) activity. L8 cells did not utilize selenium from Gpx3, the other plasma selenoprotein. Neither did they utilize it from Sepp1Δ²⁴⁰⁻³⁶¹, the isoform of Sepp1 that lacks the selenium-rich C-terminal domain. To identify Sepp1 receptors, a solubilized membrane fraction was passed over a Sepp1 column. The receptors apoER2 and Lrp1 were identified in the eluate by mass spectrometry. siRNA experiments showed that knockdown of apoER2, but not of Lrp1, inhibited ⁷⁵Se uptake from ⁷⁵Se-labeled Sepp1. Addition of protamine to the medium or treatment of the cells with chlorate also inhibited ⁷⁵Se uptake. Blockage of lysosome acidification did not inhibit uptake of Sepp1 but did prevent its digestion and thereby utilization of its selenium. These results indicate that L8 cells take up Sepp1 by an apoER2-mediated mechanism requiring binding to heparin sulfate proteoglycans. The presence of at least part of the selenium-rich C-terminal domain of Sepp1 is required for uptake. RT-PCR showed that mouse tissues express apoER2 in varying amounts. It is postulated that apoER2-mediated uptake of long-isoform Sepp1 is responsible for selenium distribution to tissues throughout the body.

INTRODUCTION

Animal cells must acquire selenium for synthesis of their selenoproteins. Some tissues tolerate selenium deficiency reasonably well, but others, e.g., testis and brain, require a reliable supply of the element to support their function and viability. In recent years, Sepp1³ has been shown to supply selenium to those high-need tissues (1,2).

Sepp1 is a complex protein with 2 major domains (3,4). The N-terminal domain, two-thirds of the primary structure, contains one selenocysteine residue in a redox motif and several potential heparin-binding sites. The shorter C-terminal domain contains 9 selenocysteine residues. Four Sepp1 isoforms that share the same N-terminal amino acid sequence, but have different termination sites, have been purified from rat plasma (5). The shortest isoform
(Sepp1Δ240-361) is the N-terminal domain that terminates at the second selenocysteine. The longest isofrom is the full-length protein and the other isofroms terminate at the third and seventh selenocysteine residues. The individual isofroms have not been quantified and the reasons for their existence are not known. There is evidence that isofroms also exist in mouse and human plasma (6,7) but they have not been characterized or quantified.

Sepp1 and Gpx3 account for greater than 97% of mouse plasma selenium but, of the two, only Sepp1 appears to have a role in selenium transport (8). Mice with deletion of Sepp1 or of both these selenoproteins survive only when fed high-selenium diet (1), indicating that small molecule forms of selenium are able to supply the element to high-need tissues when selenium supply is abundant. Sepp1, however, is needed to ensure appropriate tissue selenium distribution when selenium supply is normal or deficient.

Receptor-mediated endocytosis of Sepp1 supplies the element to tissues that depend on it for their function and survival. Spermatogenesis, a selenium-dependent process, is supported by apoER2-mediated endocytosis of Sepp1 by Sertoli cells (9). ApoER2, a member of the low-density lipoprotein receptor family, also maintains brain selenium (10). Megalin, another low-density lipoprotein receptor family member, facilitates endocytosis of filtered forms of Sepp1 by kidney proximal convoluted tubule cells (11). It has not been reported whether Sepp1 supplies other tissues with selenium through a receptor-mediated mechanism.

Although cultured Jurkat cells have been shown to take up selenium supplied as Sepp1, the mechanism of that acquisition was not presented (12). The work reported here characterizes the uptake and utilization of mouse Sepp1 selenium by the rat myoblast L8 cell line. Unexpectedly, L8 cells were found to take up Sepp1 by apoER2-mediated endocytosis, implying that apoER2 has a major role in systemic selenium physiology.

METHODS

Animals. These studies used sera from mice with deleted or altered serum selenoproteins to characterize Sepp1 uptake. The use of selenoproteins synthesized in mice was necessary because synthesis of these selenoproteins in bacterial systems has not yet been achieved. Mice used in these experiments were taken from our C57BL/6-congenic Sepp1+/− (1), Sepp1Δ240-361+/− (6), and Gpx3−/− (8) colonies. They were housed in our Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-approved facility that has a 12 h/12 h light/dark cycle. Food and tap water were provided ad libitum. The diet fed was Torula yeast-based and was formulated to our specifications (13) by Harlan Teklad (Madison, WI). It was used in the basal, selenium-deficient form and in a selenium-adequate form supplemented with 0.25 mg (1.0 mg for mice with Sepp1 deleted) selenium per kg as sodium selenite.

Care and use of animals in these experiments conformed to National Institutes of Health guidelines for animal care and use in research. The Vanderbilt Institutional Animal Care and Use Committee approved all animal protocols.

Materials. Rat myoblast L8 and cardiomyocyte H9c2 cell lines were purchased from the American Type Culture Collection (Manassas, VA). The rat monoclonal antibody 9S4 against the N-terminal domain of mouse Sepp1 was previously described (6). The hybridoma that produces 9S4 was a gift from Dr. T. Naruse of Kaketsuken, The Chemo-Sero-Therapeutic Research Institute (Kumamoto, Japan). 75Se-labeled selenite, specific activity ~2,100 μCi/μg selenium, was purchased from the University of Missouri Research Reactor Facility (Columbia, MO).

The primers, target siRNAs, and Universal Negative Control#1 siRNA (Table 1) were purchased from Sigma-Aldrich (The Woodlands, TX). High-Capacity cDNA Reverse Transcriptase Kit and 2 x Power SYBR Green PCR Master Mix were purchased from Applied Biosystems (Foster City, CA). Lipofectamine RNAiMAX, NuPAGE gels, TRIzol reagent, and NuPAGE sample buffer were purchased from Invitrogen (Carlsbad, CA). RT-PCR reagents were purchased from Applied Biosystems (Foster City, CA).

Bafilomycin A1 was purchased from Tocris Bioscience (Bristol, UK); chloroquine diphosphate and chlorpromazine were purchased from MP Biomedicals (Solon, OH); protamine was purchased from Sigma-Aldrich (St. Louis, MO); and sodium chlorate and nystatin were purchased...
from Fisher Scientific (Pittsburgh, PA). Other chemicals were reagent grade or better.

**Selenium and Gpx assays.** Selenium was determined in tissues and serum by the method of Koh and Benson (14) as modified by Sheehan and Gao (15). Gpx activity was measured in cell lysate as previously described, using 0.25 mM H₂O₂ as substrate (1). Protein concentrations were determined using bicinchoninic acid (BCA) assay reagents (Pierce Chemical, Rockford, IL).

**Selenium labeling of serum selenoproteins.** Mice were switched from selenium-adequate diet to selenium-deficient diet 2 days before intraperitoneal injection of 20 µCi of ⁷⁵Se-labeled selenite. Four hours after injection, mice were anesthetized with isoflurane and blood was collected from the vena cava and allowed to clot. Serum was separated by centrifugation and dialyzed overnight against PBS at 4ºC before being added to cell culture medium. ⁷⁵Se was quantified using a γ-counter (PerkinElmer model 1480 Wizard 3² gamma counter, Shelton, CT).

**Cell culture.** L8 and H9c2 cells were maintained in DMEM/F-12 medium containing 10% FBS at 37ºC under 95% air, 5% CO₂. All experiments were carried out in medium that did not contain FBS.

For inhibition of heparan sulfate synthesis, cells were preincubated for 24 h in serum-free medium at several concentrations of sodium chlorate. For inhibition of lysosome acidification, cells were incubated in serum-free medium containing 100 nM bafilomycin A1 or 100 nM chloroquine beginning 30 min before the start of the experiments (16,17).

**⁷⁵Se uptake determination.** L8 or H9c2 cells were trypsinized and collected by centrifugation. They were re-suspended in serum-free DMEM/F-12 medium at 3.5 x 10⁶ cells/well in a volume of 500 µL/well and cultured for 48 h before use in the ⁷⁵Se uptake assay. The cells were rinsed with PBS three times and incubated with 200 µL of DMEM/F-12, containing the ⁷⁵Se source (⁷⁵Se-labeled selenite or specific ⁷⁵Se-labeled serum), for 5 h under humidified 95% air, 5% CO₂ at 37ºC. After incubation, the supernatant was removed and the cells were washed three times with PBS and then lysed with 2% SDS in 25 mM Tris base, 192 mM glycine. ⁷⁵Se was determined in the lysate. All experiments were performed in duplicate.

**Selenium utilization determination.** Gpx activity was determined in cells as a measure of the utilization of selenium taken up by them. Cells (3.5 x 10⁶/dish) were cultured in 100 mm Petri dishes in 5 mL serum-free medium for 48 h. Then 5 mL fresh medium containing a source of selenium was added and the cells were cultured for an additional 24 h. Cells were rinsed 3 times with PBS, removed by cell scraper in 0.5 mL of lysis buffer (0.1 M phosphate buffer, pH 7.0, containing 2 mM EDTA and 2 mM sodium azide), lysed by sonication, and centrifuged at 12,000 g for 15 min at 4ºC. Gpx activity and total protein concentration of the supernatant were determined.

To ensure that equivalent amounts of selenium were added in the form of different selenoproteins, selenium concentrations were determined in dialyzed sera from Sepp1⁺/⁺, Gpx3⁺/⁺, and Sepp1Δ²₄₀-3₆₁/Gpx3⁺/⁺ mice. Selenoprotein sources of selenium were Sepp1 in Gpx3⁻/⁻ serum containing 328 ng selenium/mL (4150 nM selenium); Sepp1Δ²₄₀-3₆₁ in Sepp1Δ²₄₀-3₆₁/Gpx3⁻/⁻ serum containing 114 ng selenium/mL (1440 nM selenium); and Gpx3 in Sepp1⁻/⁻ serum containing 52 ng selenium/mL (658 mM selenium).

To add a source of selenium equivalent in concentration to the 83 nM selenium as Sepp1 in medium containing 2% Gpx3⁻/⁻ serum, serum containing only Gpx3 (Sepp1⁻/⁻ serum) was added as 15% of the medium to give 99 nM selenium in the medium and Sepp1Δ²₄₀-3₆₁ (Sepp1Δ²₄₀-3₆₁/Gpx3⁻/⁻ serum) was added as 7% of the medium to give 101 nM selenium in the medium. Sodium selenite was added to the medium in the concentrations indicated.

**RNAi experiments.** Transfections with siRNAs were performed with Lipofectamine RNAiMAX according to the procedure recommended by the manufacturer. Cells were transfected in the presence of Universal Negative Control#1 siRNA or siRNA specific for rat Lrp1 or apoER2 (Table 1). Briefly, a solution containing 1.2 µL siRNA (20 µM) or 1.2 µL negative control siRNA (20 µM), 1.5 µL Lipofectamine RNAiMAX, and 200 µL serum-free medium was incubated at room temperature for 20 min. One mL of cell suspension (7 x 10⁵ cells/mL in serum-free medium) was added to the siRNA solution and then it was plated in 4 wells at 300 µL (1.75 x 10⁶ cells) per well. It was incubated in humidified 95% air, 5% CO₂ at 37ºC.
Sepp1 supplies selenium to a muscle cell line via apoER2 for 4 h, then 600 µL of serum-free medium was added to each well. Incubation was continued for 44 additional hours. Silencing was then assessed by measuring levels of Lrp1 and apoER2 mRNA using RT-PCR. Other cells were used in 75Se-labeled Sepp1 uptake experiments.

Affinity Purification and Identification of Sepp1-binding Proteins by Mass Spectrometry. Preparation of a rat Sepp1 column and purification of Sepp1-binding proteins were described previously (9). Briefly, a membrane preparation was prepared from L8 cells. Trypsinized cells were washed with PBS, suspended in ice-cold TNI (150 mM NaCl, 25 mM Tris-HCl, pH 7.5, 2 mM benzamidine, 1 µg/mL leupeptin, 1 µg/mL pepstatin, 1 mM sodium fluoride, 1 mM sodium vanadate, and 0.05% sodium azide) and homogenized with a Polytron homogenizer. The suspension was centrifuged at 11,000 g for 30 min; the supernatant was discarded; and the pellet was washed by resuspension in TNI followed by repeat centrifugation. Membrane proteins were solubilized at 4°C in TNI containing 0.2% Triton X-100 and insoluble material was pelleted under the same centrifugation conditions as above. The Triton-soluble fraction was adjusted to 1 mM each CaCl₂ and MgCl₂ and then incubated with the AminoLink resin with bound rat Sepp1. The resin was packed into a column and washed with 10 volumes of TNI followed by 10 volumes of 1 M NaCl, 1 mM Tris-HCl, pH 7.5. Bound proteins were eluted with 0.1 M glycine-HCl, pH 2.5. Proteins in the glycine-eluted fraction were trypsin-digested into peptides that were subsequently analyzed using multidimensional high performance liquid chromatography-coupled mass spectrometry (MudPIT)(18). Peptide tandem mass spectra were acquired in a data dependent manner over 16 h (8 MudPIT cycles) using a Thermo-Fisher LTQ. These spectra were queried against a UniProt rodent database using SEQUEST (19) and resulting identifications were filtered and collated by protein using IDPicker (20).

SDS-PAGE and autoradiography. L8 cells were incubated with 75Se-labeled Sepp1 for 5 h in the presence or absence of 100 µM chloroquine. Cells were washed 3 times with PBS and lysed with PBS containing 0.2% Triton X-100. Aliquots of lysate were used for SDS-PAGE and for immunoaffinity purification of Sepp1. 0.5 mL of PBS, 0.2% Triton X-100 was added to 100 µL of lysate and the mixture was incubated for 1 hour with anti-mouse Sepp1 (9S4) conjugated with 50 µL of AminoLink beads. The beads were pelleted by centrifugation at 1500 g for 1 min and the pellet was washed 3 times with 1 mL PBS each time. The final pellet was incubated in SDS-PAGE sample buffer heated to 100°C for 5 min and then centrifuged. The supernatant fraction collected was ~30 µL and 25 µL was used for SDS-PAGE and autoradiography. 1 µL 75Se-labeled Sepp1 mouse serum was applied as a control. For the autoradiograph used to visualize selenoproteins in L8 cells, SDS-PAGE was performed using a 4-12% Bis-Tris NuPAGE Novex acrylamide gel as described previously (9). The dried gel was exposed to Kodak XAR film for 7 d before the film was developed.

RT-PCR. Cells rinsed with PBS or tissues pulverized under liquid nitrogen were treated with TRIzol Reagent. Bone marrow cells were obtained from femur and tibia (21). Total RNA was isolated according to the manufacturer’s protocol. RNA concentration was determined by measurement of A₂₆₀. cDNA was made from total RNA by High-Capacity cDNA Reverse Transcriptase Kit with 0.5 µg total RNA per 20 µL reaction following the manufacturer's instructions. Quantitative PCR for gene expression was performed with 5 µL diluted cDNA using Power SYBR Green PCR Master Mix with specific primer concentration at 250 nM in a total reaction volume of 20 µL. StepOnePlus Real-Time PCR System and StepOne software V2.1 (Applied Biosystems) were used to collect and analyze data. Three replicates of each sample were amplified. For cell experiments, relative quantitation of RNA levels was determined by construction of relative standard curves. The control cDNA for these curves was made from total RNA isolated from cells treated with Universal Negative Control#1 siRNA. Relative standard curves were generated for each gene by serial 1:5 dilutions of the control cDNA to give a final dilution of 1:3125. cDNA from siRNA-treated cells (1:125 dilutions) were amplified simultaneously with control cDNA (1:125 dilution). Primers for amplification of rat Lrp1, apoER2 and cyclophilin A (Ppia) (Table 1) were used. Ppia served as the endogenous control. The target mRNA quantity in each siRNA-treated sample was determined from the relative standard
Sepp1 supplies selenium to a muscle cell line via apoER2

curve (using sample CT values) and expressed in arbitrary units (RQ). For tissue experiments, relative quantitation of RNA levels was determined by comparative CT reactions (ΔΔCT analysis). Primers for amplification of mouse apoER2 and hypoxanthine phosphoribosyltransferase (Hprt) (Table 1) were used. Hprt served as the endogenous control. The synthesized cDNA was diluted 1:125 prior to use. The target mRNA quantity in each tissue was expressed in arbitrary units (RQ).

Statistics

Statistical comparisons between groups were made on an iMac using Prism 5 for Macintosh Version 5.00.2 software program (GraphPad Software, Inc.). Tukey’s Multiple Comparison Test was applied after analysis by 1-way ANOVA. Where appropriate, Student’s t-test was used to compare groups. Groups were considered to be significantly different when p<0.05.

RESULTS

Uptake of Sepp1 and utilization of its selenium

Uptake of selenium from the culture medium and utilization of it for Gpx synthesis were studied in the rat myoblast L8 cell line. L8 cells took up 75Se in a time-dependent manner from mouse Gpx3−/− serum that contained 75Se-labeled Sepp1 as the only selenoprotein (Fig 1A). We chose 5 h as the endpoint for subsequent 75Se-Sepp1 uptake studies.

Inhibition of 75Se uptake by unlabeled serum selenoproteins was assessed. Unlabeled Gpx3−/− serum, which contained 75Se-labeled Sepp1 as the only selenoprotein (Fig 1A). We chose 5 h as the endpoint for subsequent 75Se-Sepp1 uptake studies.

Route of Sepp1 uptake

Sepp1 is a heparin-binding protein (22). We evaluated the requirement of that property for 75Se uptake by L8 cells. When heparin was added to the medium, 75Se uptake from 75Se-labeled Sepp1, but not from 75Se-labeled selenite, was inhibited (Figs 3A and 3D). This result is compatible with heparin masking the heparin-binding sites of Sepp1 and preventing it from binding to HSPGs on the cell surface. Adding protamine, a compound that binds to heparin and to HSPGs, also inhibited 75Se uptake from 75Se-labeled Sepp1 (Fig 1B). It had a slight effect on uptake of 75Se from 75Se-labeled selenite (Fig 3E). Chlorate treatment of cells inhibits heparan sulfate maturation and depletes cell-surface HSPGs. Chlorate inhibited 75Se uptake from 75Se-labeled Sepp1 in a dose-dependent manner (Fig 3C) but had only a slight effect on uptake of 75Se from 75Se-labeled selenite (Fig 3F).

We sought Sepp1 receptors in membranes of L8 cells by preparing a solubilized membrane fraction and passing it over a column with rat Sepp1 bound to the beads. The eluted fraction was analyzed by mass spectrometry. Table 2 shows that apoER2 peptides with 22% coverage of the apoER2 sequence and Lrp1 peptides with 18% coverage of its sequence were identified. Megalin peptides were not detected. This indicates that apoER2 and Lrp1, both members of the low-density lipoprotein receptor family, were present in L8 cells and that they bound to the Sepp1 column. Thus, either of these receptors or both of them might be responsible for Sepp1 uptake.

We employed siRNA techniques to determine the role of these receptors in Sepp1 uptake. Cells
Sepp1 supplies selenium to a muscle cell line via apoER2

were prepared in which each receptor was knocked down and in which both were knocked down simultaneously. Figure 4A shows that the mRNAs were knocked down to less than 31% of control. Knock down of Lrp1 had no effect on uptake of \(^{75}\)Se from \(^{75}\)Se-labeled Sepp1 (Fig 4B). Knock down of apoER2 inhibited \(^{75}\)Se uptake to 56% of the negative siRNA control and knock down of both receptors had the same effect as knock down of apoER2 alone. This implicates apoER2 in facilitating uptake by L8 cells of the selenium supplied as Sepp1.

The same experimental protocol was carried out using H9c2 cells, a cell line derived from embryonic BD1X rat heart tissue. Figure 4C shows that the mRNA of Lrp1 was knocked down to 22% of control and that of apoER2 to 25%. Uptake of \(^{75}\)Se from \(^{75}\)Se-labeled Sepp1 was not decreased by knock down of Lrp1 but it was decreased to 18% of control by knock down of apoER2 and to 31% by knock down of both receptors simultaneously (Fig 4D). Thus, apoER2, but not Lrp1, mediated uptake of Sepp1 selenium in this line of cardiac myoblasts.

Endocytosis takes place by several pathways so we investigated the roles of clathrin-dependent and caveolin-dependent pathways. Figure 5A shows that chlorpromazine inhibited uptake of \(^{75}\)Se from \(^{75}\)Se-labeled Sepp1, suggesting that the process is clathrin dependent. Nystatin, an inhibitor of the caveolin-dependent pathway, had no (or minimal) effect (Fig 5B). ApoER2 has been reported to be endocytosed by the clathrin-dependent pathway so this result was not unexpected (23).

Lysosomal digestion of Sepp1

Ligands of low-density lipoprotein receptors are usually delivered to lysosomes for digestion. Chloroquine and bafilomycin A1 both inhibit lysosome acidification and thereby prevent digestion of proteins inside the lysosome (16,17). Figures 6A and 6B show that adding these inhibitors to the medium blocked development of Gpx activity after the addition of serum containing Sepp1. The inhibitors did not prevent uptake of \(^{75}\)Se from \(^{75}\)Se-labeled Sepp1, however (Fig 6C).

We examined L8 cells that had been cultured with \(^{75}\)Se-labeled Sepp1 in the presence of chloroquine to determine whether they contained undigested Sepp1. Lysed cells and Sepp1 that had been immunoaffinity purified from lysed cells were subjected to SDS-PAGE and \(^{75}\)Se was detected by autoradiography (Fig 6D). Immunoaffinity purification was carried out with the monoclonal antibody 9S4 that recognizes an epitope in the N-terminal domain of Sepp1. Lane 5 represents serum, containing \(^{75}\)Se-labeled Sepp1, included to indicate the migration of the \(^{75}\)Se-labeled Sepp1 that had been added to the culture medium. Chloroquine addition to the medium caused accumulation of apparently undigested \(^{75}\)Se-labeled Sepp1 in cells (compare lane 2 with lane 1). In the absence of chloroquine (lane 3) the only \(^{75}\)Se-labeled Sepp1 form detected that bound to the antibody 9S4 was a fragment that migrated at 18 kDa, but in the presence of chloroquine (lane 4) the only \(^{75}\)Se-labeled Sepp1 form that was visible migrated near the undigested serum \(^{75}\)Se-labeled Sepp1 (lane 5). These results indicate that lysosomal digestion is necessary for utilization of the selenium in endocytosed Sepp1.

ApoER2 mRNA expression in muscle and other tissues

ApoER2 mRNA is expressed at high levels in testis and brain (24). The results in table 2 and figure 4 show that the rat L8 and H9c2 myoblast cell lines express apoER2 also. We carried out RT-PCR of several adult mouse tissues to determine the relative expression of apoER2 mRNA in them (Fig 7). Expression by testis, bone marrow, placenta, brain, and thymus was greater than by spleen, quadriceps, and heart. Expression by quadriceps muscle was 1.4% of expression by brain and 0.07% of expression by testis. ApoER2 mRNA was questionably present in kidney and liver. These results show that apoER2 is widely expressed in the body.

DISCUSSION

The results presented here demonstrate that L8 muscle cells take up Sepp1 and utilize its selenium for selenoprotein synthesis. The uptake was inhibited by interference with binding to HSPGs and by knockdown of apoER2. After uptake of Sepp1 by the cell, utilization of its selenium required lysosomal digestion. ApoER2 mRNA expression was shown to occur in many tissues. These findings outline the process by which Sepp1 supplies selenium to an individual cell and have implications for the supply of selenium to cells throughout the body.
L8 cells take up selenium from ‘all isoform’ Sepp1 (Fig 1). Neither the shortest isoform of Sepp1, Sepp1Δ240-361, nor Gpx3 inhibits that uptake or provides selenium to the cells (Figs 1 and 2). Thus, of the plasma selenoproteins, only one or more of the longer Sepp1 isoforms is (are) taken up. None of these putative mouse isoforms has been characterized but all of them would contain some of the C-terminal domain, implying that recognition of the selenium-rich C-terminal domain of Sepp1 is necessary for the uptake. This in vitro finding complements observations in vivo that apoER2-mediated endocytosis of Sepp1Δ240-361 does not occur in the testis or placenta.4,5

Another requirement for uptake of Sepp1 appears to be binding to HSPGs (Fig 3). Receptor-mediated processes often involve binding of the ligand to cell surface HSPGs (25). Such binding can serve as a reservoir for the ligand and can facilitate interaction of the ligand with its receptor. Heparin-binding sites have been identified only in the N-terminal domain of Sepp1 (22), but C-terminal fragments of human Sepp1 have also been shown to bind to heparin (26). Thus, binding of Sepp1 to cell-surface HSPGs might occur through either the N-terminal domain or the C-terminal domain. This leaves open the possibility that any Sepp1 form that contains the selenium-rich C-terminal domain could be taken up by the mechanism under study. However, we presented to our cells only forms that contained the N-terminal domain so our results do not allow us to reach the conclusion that the N-terminal domain is not needed for uptake by L8 cells. Other possibilities remain, including that the HSPG interaction takes place through the N-terminal domain and the apoER2 binding through the C-terminal domain.

Receptor-mediated uptake of Sepp1 has been previously observed in only a few tissues and those observations were made in vivo. ApoER2 facilitates Sepp1 uptake from the circulation by testis and brain (9,10), and megalin facilitates Sepp1 uptake by proximal convoluted tubule cells from the urinary filtrate (11). There is evidence that these two receptors mediate uptake of different forms of Sepp1. Megalin mediates Sepp1 uptake in Sepp1Δ240-361/AΔ240-361 mice and apoER2 does not (11). This indicates that the megalin uptake process, unlike that of apoER2, does not require the presence of the Sepp1 C-terminal domain. Also, the Sepp1 forms in urinary filtrate that are taken up by megalin appear to be smaller than the forms in the systemic circulation (27). Taken together, these observations suggest that apoER2 uptake of Sepp1 from the systemic circulation has the purpose of acquiring selenium for the cell via the selenium-rich C-terminal domain, while megalin uptake, which is from the urinary filtrate, has the two-fold purpose of scavenging selenium-containing fragments of Sepp1 to prevent their loss in the urine and of supplying selenium to the proximal convoluted tubule cells for synthesis of Gpx3 (28).

In spite of its binding to a Sepp1 column, knock down of Lrp1 did not impair uptake of Sepp1 by muscle cells (Fig 4). Thus, apoER2 is the only receptor presently known to mediate endocytosis of Sepp1 from the systemic circulation. Our demonstration that apoER2 is expressed in a number of tissues (Fig 7) allows the speculation that apoER2-mediated endocytosis of Sepp1 occurs in many tissues of the body. Regulation of Sepp1 uptake by each tissue might be determined by expression of apoER2 by the tissue (Fig 7) and/or by other factors, such as binding to cell-surface HSPGs.

Based on the results presented here, we postulate that apoER2-mediated endocytosis of Sepp1 ensures the supply of selenium to muscle cells (and to other cells that express this receptor) under selenium-deficiency conditions, just as it has been shown to do in the brain (10). It has not been possible to test this directly because the production of selenium deficiency in apoER2−/− mice leads rapidly to neurological dysfunction and death before selenium deficiency can be established in the whole animal. However, comparison of tissue selenium concentrations in wild-type mice that were severely selenium deficient with concentrations in selenium-replete mice revealed that retention in brain was 56%, testis 22%, muscle 15%, kidney 9%, and liver 3% (4). Figure 7 shows that brain and testis had relatively high expressions of apoER2 mRNA. Muscle expressed a significant amount of apoER2 mRNA but kidney and liver did not. Kidney, however, expresses megalin and that might account for its retention of more selenium than liver. These correlations between selenium retention and apoER2 mRNA expression support a role for apoER2 in maintaining selenium supply to...
many tissues under conditions of selenium deficiency.

In conclusion, the finding that apoER2 mediates uptake of Sepp1 by two muscle cell lines and the recognition that many tissues express apoER2 suggest that Sepp1 uptake by apoER2-mediated endocytosis is responsible for maintaining the physiological distribution of selenium in the body under varying conditions of selenium supply.
REFERENCEs

1. Hill, K. E., Zhou, J., McMahan, W. J., Motley, A. K., Atkins, J. F., Gesteland, R. F., and Burk, R. F. (2003) Deletion of selenoprotein P alters distribution of selenium in the mouse. *J. Biol. Chem.* **278**, 13640-13646

2. Schomburg, L., Schweizer, U., Holtmann, B., Flohé, L., Sendtner, M., and Kohrle, J. (2003) Gene disruption discloses role of selenoprotein P in selenium delivery to target tissues. *Biochem. J.* **370**, 397-402

3. Burk, R. F., and Hill, K. E. (2005) Selenoprotein P: An extracellular protein with unique physical characteristics and a role in selenium homeostasis. *Annu. Rev. Nutr.* **25**, 215-235

4. Burk, R. F., and Hill, K. E. (2009) Selenoprotein P-Expression, functions, and roles in mammals. *Biochim. Biophys. Acta* **1790**, 1441-1447

5. Ma, S., Hill, K. E., Caprioli, R. M., and Burk, R. F. (2002) Mass spectrometric characterization of full-length rat selenoprotein P and three isoforms shortened at the C terminus. Evidence that three UGA codons in the mRNA open reading frame have alternative functions of specifying selenocysteine insertion or translation termination. *J. Biol. Chem.* **277**, 12749-12754

6. Hill, K. E., Zhou, J., Austin, L. M., Motley, A. K., Ham, A. J., Olson, G. E., Atkins, J. F., Gesteland, R. F., and Burk, R. F. (2007) The selenium-rich C-terminal domain of mouse selenoprotein P is necessary for supply of selenium to brain and testis but not for maintenance of whole-body selenium. *J. Biol. Chem.* **282**, 10972-10980

7. Meplan, C., Nicol, F., Burtle, B. T., Crosley, L. K., Arthur, J. R., Mathers, J. C., and Hesketh, J. E. (2009) Relative abundance of selenoprotein P isoforms in human plasma depends on genotype, Se intake, and cancer status. *Antioxid. Redox Signal* **11**, 2631-2640

8. Olson, G. E., Whitin, J. C., Hill, K. E., Winfrey, V. P., Motley, A. K., Austin, L. M., Deal, J., Cohen, H. J., and Burk, R. F. (2010) Extracellular glutathione peroxidase (Gpx3) binds specifically to basement membranes of mouse renal cortex tubule cells. *Am. J. Physiol. Renal Physiol.* **298**, F1244-F1253

9. Olson, G. E., Winfrey, V. P., Nagdas, S. K., Hill, K. E., and Burk, R. F. (2007) Apolipoprotein E receptor-2 (ApoER2) mediates selenium uptake from selenoprotein P by the mouse testis. *J. Biol. Chem.* **282**, 12290-12297

10. Burk, R. F., Hill, K. E., Olson, G. E., Weeber, E. J., Motley, A. K., Winfrey, V. P., and Austin, L. M. (2007) Deletion of apolipoprotein E receptor-2 in mice lowers brain selenium and causes severe neurological dysfunction and death when a low-selenium diet is fed. *J. Neurosci.* **27**, 6207-6211

11. Olson, G. E., Winfrey, V. P., Hill, K. E., and Burk, R. F. (2008) Megalin mediates selenoprotein P uptake by kidney proximal tubule epithelial cells. *J. Biol. Chem.* **283**, 6854-6860

12. Saito, Y., and Takahashi, K. (2002) Characterization of selenoprotein P as a selenium supply protein. *Eur. J. Biochem.* **269**, 5746-5751

13. Hill, K. E., Zhou, J., McMahan, W. J., Motley, A. K., and Burk, R. F. (2004) Neurological dysfunction occurs in mice with targeted deletion of selenoprotein P gene. *J. Nutr.* **134**, 157-161

14. Koh, T. S., and Benson, T. H. (1983) Critical re-appraisal of fluorometric method for determination of selenium in biological materials. *J. Assoc. Off. Anal. Chem.* **66**, 918-926

15. Sheehan, T. M. T., and Gao, M. (1990) Simplified fluorometric assay of total selenium in plasma and urine. *Clin. Chem.* **36**, 2124-2126

16. Goldstein, J. L., Brunschede, G. Y., and Brown, M. S. (1975) Inhibition of proteolytic degradation of low density lipoprotein in human fibroblasts by chloroquine, concanavalin A, and Triton WR 1339. *J. Biol. Chem.* **250**, 7854-7862

17. Yoshimori, T., Yamamoto, A., Moriyama, Y., Futai, M., and Tashiro, Y. (1991) Bafilomycin A1, a specific inhibitor of vacuolar-type H(+) -ATPase, inhibits acidification and protein degradation in lysosomes of cultured cells. *J. Biol. Chem.* **266**, 17707-17712
Sepp1 supplies selenium to a muscle cell line via apoER2

18. MacCoss, M. J., McDonald, W. H., Saraf, A., Sadygov, R., Clark, J. M., Tasto, J. J., Gould, K. L., Wolters, D., Washburn, M., Weiss, A., Clark, J. I., and Yates, J. R. 3rd. (2002) Shotgun identification of protein modifications from protein complexes and lens tissue. Proc. Natl. Acad. Sci. U.S.A. 99, 7900-7905

19. Yates, J. R., 3rd, Eng, J. K., McCormack, A. L., and Schieltz, D. (1995) Method to correlate tandem mass spectra of modified peptides to amino acid sequences in the protein database. Anal. Chem. 67, 1426-1436

20. Ma, Z.-Q., Dasari, S., Chambers, M. C., Litton, M. D., Sobecki, S. M., Zimmerman, L. J., Halvey, P. J., Schilling, B., Drake, P. M., Gibson, B. W., and Taub, D. L. (2009) IDPicker 2.0: Improved protein assembly with high discrimination peptide identification filtering. J. Proteome Res. 8, 3872-3881

21. Muccioli, M., Pate, M., Omosebi, O., and Benencia, F. (2011) Generation and labeling of murine bone marrow-derived dendritic cells with Qdot nanocrystals for tracking studies. J. Vis. Exp. 52, e2785

22. Hondal, R. J., Ma, S., Caprioli, R. M., Hill, K. E., and Burk, R. F. (2001) Heparin-binding histidine and lysine residues of rat selenoprotein P. J. Biol. Chem. 276, 15823-15831

23. Cuitino, L., Matute, R., Retamal, C., Bu, G., Inestrosa, N. C., and Marzolo, M. P. (2005) ApoER2 is endocytosed by a clathrin-mediated process involving the adaptor protein Dab2 independent of its Rafts' association. Traffic 6, 820-838

24. Kim, D. H., Iijima, H., Goto, K., Sakai, J., Ishii, H., Kim, H. J., Suzuki, H., Kondo, H., Saeki, S., and Yamamoto, T. (1996) Human apolipoprotein E receptor 2. A novel lipoprotein receptor of the low density lipoprotein receptor family predominantly expressed in brain. J. Biol. Chem. 271, 8373-8380

25. Sarrazin, S., Lamanna, W. C., and Esko, J. D. (2011) Heparan sulfate proteoglycans. Cold Spring Harbor perspectives in biology 3 PMID:21690215

26. Hirashima, M., Naruse, T., Maeda, H., Nozaki, C., Saito, Y., and Takahashi, K. (2003) Identification of selenoprotein P fragments as a cell-death inhibitory factor. Biol. Pharmaceutical Bulletin 26, 794-798

27. Chiu-Ugalde, J., Theilig, F., Behrends, T., Drebes, J., Sieland, C., Subbarayal, P., Kohrle, J., Hammes, A., Schomburg, L., and Schweizer, U. (2010) Mutation of megalin leads to urinary loss of selenoprotein P and selenium deficiency in serum, liver, kidneys and brain. Biochem. J. 431, 103-111

28. Avissar, N., Ornt, D. B., Yagil, Y., Horowitz, S., Watkins, R. H., Kerl, E. A., Takahashi, K., Palmer, I. S., and Cohen, H. J. (1994) Human kidney proximal tubules are the main source of plasma glutathione peroxidase. Am. J. Physiol. 266, C367-C375
**Acknowledgements**—The authors are grateful to Teri D. Stevenson for husbandry of the mice and to Amy K. Motley for performing the selenium assays.

**FOOTNOTES**

*This work was supported by NIH grants R37 ES02497, 5P30 DK058404, and 5P30 ES000267.

1 To whom correspondence may be addressed: Division of Gastroenterology, Hepatology, and Nutrition, Department of Medicine, 1030C Medical Research Building IV, Vanderbilt Medical Center, Nashville, TN 37232-0252, Tel.: 615-343-4748, Fax: 615-343-6229, E-mail: raymond.burk@vanderbilt.edu

2 Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN

3 Abbreviations: apoER2, apolipoprotein E receptor-2; Ppia, cyclophilin A; DMEM, Dulbecco’s minimal essential medium; FBS, fetal bovine serum; Gpx, glutathione peroxidase; Gpx3, glutathione peroxidase-3; HSPG, heparin sulfate proteoglycan; Hprt, hypoxanthine phosphoribosyltransferase; Lrp1, low density lipoprotein receptor-related protein; PBS, phosphate buffered saline; RT-PCR, Real-time polymerase chain reaction; RQ, relative quantitation; Sepp1, selenoprotein P; siRNA, small interfering RNA; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis

4 R.F. Burk, G.E. Olson, K.E. Hill, V.P. Winfrey, A.K. Motley, and S. Kurokawa, Mechanisms of selenoprotein P-dependent maternal-fetal selenium transfer, manuscript in preparation.

5 R.F. Burk, G.E. Olson, K.E. Hill, V.P. Winfrey, unpublished observation.

**FIGURE LEGENDS**

**Figure 1.** Uptake of $^{75}$Se from $^{75}$Se-labeled Sepp1 by L8 cells and inhibition of that uptake by serum selenoproteins. L8 cells were incubated in medium containing 2% Gpx3$^{-/-}$ mouse serum that contained $^{75}$Se-labeled Sepp1 as the only selenoprotein. (A) The $^{75}$Se content of the cells was determined at intervals of up to 10 h incubation. (B) The effect of non-labeled Sepp1 on uptake of $^{75}$Se from $^{75}$Se-labeled Sepp1 was determined. $^{75}$Se uptake was determined in cells cultured for 5 h with Gpx3$^{-/-}$ mouse serum added to the medium in different concentrations. (C) The effect of non-labeled Gpx3 and the shortest isoform of Sepp1 (Sepp1$^{Δ240-361}$) on uptake of $^{75}$Se from $^{75}$Se-labeled Sepp1 was determined. $^{75}$Se uptake was determined in cells cultured for 5 h with Sepp1$^{Δ240-361}$ mouse serum added to the medium in different concentrations. All values are means (n=4) with 1 S.D. indicated by the bracket. Asterisks indicate values significantly different (p<0.05) from the 30 min value (panel A) or the value with no unlabeled serum added (panels B and C) by Tukey’s Multiple Comparison Test applied after 1-way ANOVA of the groups.

**Figure 2.** Response of L8 cell Gpx activity to the addition of different selenium sources to the medium. Selenium-depleted cells were cultured for 24 h with a selenium source and were then assayed for Gpx activity. (A) Selenite at different concentrations was the selenium source. (B) Selenium sources were 100 nM selenite and serum that contained single selenoproteins: Sepp1 (83 nM selenium), Gpx3 (98 nM selenium), and Sepp1$^{Δ240-361}$ (101 nM selenium). The values shown are means of 3 experiments carried out in duplicate with 1 S.D. indicated by the bracket. The asterisks in (A) indicate values significantly different (p<0.05) from the value with no selenite added by Tukey’s Multiple Comparison Test applied after 1-way ANOVA of the groups and in (B) the values that were significantly different (p<0.05) from the basal value by Student’s t-test.

**Figure 3.** Influence of heparin-binding properties on L8 cell $^{75}$Se uptake from $^{75}$Se-labeled Sepp1. L8 cells were cultured for 5 h with 2% Gpx3$^{-/-}$ mouse serum that contained ‘all-isoform’ $^{75}$Se-labeled Sepp1 as the only selenoprotein (panels A, B, and C) or $^{75}$Se-labeled selenite at 100 nM (panels D, E, and F). (A and D) Heparin was added to the medium at the concentrations indicated. (B and E) Protamine was added to the medium at the concentrations indicated. (C and F) Chlorate was added to the medium at the
concentrations indicated 24 h before the $^{75}$Se source was introduced. Values shown are means (n=4) with 1 S.D. indicated by the bracket. Asterisks indicate values significantly different (p<0.05) from the initial value by Tukey’s Multiple Comparison Test applied after 1-way ANOVA of the groups.

**Figure 4.** Effect of knock down of receptors apoER2 and/or Lrp1 on $^{75}$Se uptake from $^{75}$Se-labeled Sepp1 by L8 and H9c2 cells. (A) L8 cells were cultured for 48 h with siRNA and then relative mRNA levels were determined. Values are means (n=3) with 1 S.D. indicated by the bracket. The asterisk indicates values different from control siRNA (p<0.05) by Student’s t-test. (B) Uptake in 5 h incubations of $^{75}$Se by L8 cells with knock down of receptors. Values are means of 4 experiments done in duplicate with 1 S.D. indicated by the bracket. Asterisks indicate that control was different (p<0.05) from apoER2 and double knock downs by Student’s t-test. (C) H9c2 cells were cultured for 48 h with siRNA and relative mRNA levels were determined. Values are means (n=3) with 1 S.D. indicated by the bracket. The asterisk indicates values different (p<0.05) from control siRNA by Student’s t-test. (D) Uptake in 5 h incubations of $^{75}$Se by H9c2 cells with knock down of receptors. Values are means of 4 experiments done in duplicate with 1 S.D. indicated by the bracket. The asterisk indicates that control is different (p<0.05) from apoER2 and double knock downs by Student’s t-test.

**Figure 5.** Effect of inhibiting clathrin and caveolin endocytosis pathways on the uptake of $^{75}$Se from $^{75}$Se-labeled Sepp1. L8 cells were incubated for 30 min in serum-free medium containing the indicated concentrations of (A) chlorpromazine or (B) nystatin before serum that contained $^{75}$Se-labeled Sepp1 was added. After 5 h, cells were washed and $^{75}$Se was determined in them. Values are means (n=4) with 1 S.D. indicated by the bracket. Asterisks indicate values significantly different (p<0.05) from the initial value by Tukey’s Multiple Comparison Test applied after 1-way ANOVA of the groups.

**Figure 6.** Requirement of lysosomal function for L8 cell utilization of Sepp1 selenium. (A) Effect of 100 µM chloroquine on Gpx activity in L8 cells cultured 24 h with Gpx3$^{-/-}$ serum containing Sepp1 (98 nM selenium) or with selenite (100 nM selenium). (B) Effect of 100 nM bafilomycin A1 on Gpx activity in L8 cells cultured with Gpx3$^{-/-}$ serum containing Sepp1 (98 nM selenium) or with selenite (100 nM selenium). (C) $^{75}$Se uptake by L8 cells with bafilomycin A1 (100 nM) or chloroquine (100 µM) added to the medium. Values in (A), (B), and (C) are means (n=3 experiments carried out on duplicate plates) with 1 S.D. indicated by the bracket. Treated values were not significantly different from the control values (p<0.05) by Student’s t-test. (D) Effect of chloroquine addition on Sepp1 form in L8 cells. L8 cells were incubated for 5 h with serum containing $^{75}$Se-labeled Sepp1 with or without 100 µM chloroquine. After washing, cells were lysed with SDS-PAGE buffer. Whole-cell lysates were subjected to SDS-PAGE and autoradiography (lanes 1 and 2) or to purification of Sepp1 using 9S4 antibody before the eluate was subjected to SDS-PAGE and autoradiography (lanes 3 and 4). Lane 5 shows an autoradiogram of 1 µL serum from a Gpx3$^{-/-}$ mouse that had been injected with $^{75}$Se-labeled selenite.

**Figure 7.** Relative abundance of apoER2 mRNA in tissues of mice fed diet supplemented with 0.25 mg selenium/kg as selenite. Total RNA was extracted from designated tissues and used for cDNA synthesis and real-time PCR analysis. Levels of each tissue RNA are shown relative to hprt. The brain value was set as 1 and the quantitation of each tissue is relative to it. Values are means (n=3) with the bracket indicating 1 S.D. The panel on the right shows tissues with very low relative mRNA expression (less than 3% of brain).
### TABLES

Table 1. Primer sequences used for RT-PCR quantitation

| Genes     | PCR primer sequences (5’-3’)                                                                 | GenBank accession number |
|-----------|---------------------------------------------------------------------------------------------|-------------------------|
| **A. rat cell lines**                                                   |                                                                            |
| apoER2    | GCGTTTGTACTGGGTGGGACT                                                                     | NM_009155               |
|           | GAAAATGGCCTCATTCTCCA                                                                     |                         |
| Lrp1      | GTGAGGGGTGGGTTCTTTTTC                                                                    | NM_001130490            |
|           | ATTCCAACAGCCAGTGACGT                                                                     |                         |
| Ppia      | CCCACCGTGTCTTCGACAT                                                                     | NM_017101               |
|           | CCTGTCTGCAAACAGGCTCAAA                                                                    |                         |
| **B. mouse tissues**                                                   |                                                                            |
| apoER2    | AGATGGGGCTCAACAGTCACC                                                                     | NM_001080926            |
|           | AGTGGGCGATCATAGTTGCT                                                                     |                         |
| Hprt      | TCCTCCTCACCCCGCTTTT                                                                      | NM_013556               |
|           | CCTGGTTTCATCGCTAATC                                                                      |                         |
Table 2. Mass spectrometric identification of L8 cell Sepp1-binding proteins

|                | A. Lrp1 peptides | B. apoER2 peptides |
|----------------|------------------|--------------------|
| DCPDSDEAEPECQSK| SIHLSDER         |                    |
| NGDTCVTLDDLEYLPK| NLNAPVQPFEDEPHMK|                    |
| VFFTDYGQIPK    | NVIALAFDYR       |                    |
| SGFSLGSDGK     | AGTSGPQTPNR      |                    |
| DIFVTSK        | IFSDDIHGFNIQQINDDSGER |                 |
| LYWVDADFYDR    | TTVENVNGVEGLAYHR |                    |
| SERPPIFEIR     | GWDTLYWTSYTTTITR |                    |
| MYDAQQQQVGTNK   | ETVITMSGDDHPR    |                    |
| CININWR         | AALSFGNVLTLIEK   |                    |
| LDGLCIPLR      | ILQEDFTCR        |                    |
| CSCYEGWVLEPDGESC| DKSDEKPSYCNSR   |                    |
| TTLLAGDIEHPR    | TACGVGEFR        |                    |
| DGILFWTDWDASLPR| FCSEAQFECQNR     |                    |
| IEAASMSGAGR     | CVAEALLCNQDDCDGDSER |                |
| ETGSGGWPNGLTVDYLEK | GCHVNECLSR |       |
| ILWIDAR         | KLSGCQDCEDLK     |                    |
| YDGSGHMEVLR     | CLCVEGYPAPR      |                    |
| TNTQFDLQVYHPSR  | AVTDEEFPFIFANR   |                    |
| VYWSDVR         | QGLNNAVLDFDYSR   |                    |
| NLFWTSYDTNKK    | TGLSNPDLAVWDVVGNLYWCDK |        |
| GPVGLAIDFPESK   | IGMDSGER         |                    |
| CNLDGSELEVIMTR  | ITWPNGLTVDYVTER  |                    |
| ATALAIMGDK      | IYWADAR          |                    |
| LWWADQVSEK      | EDYIEFASLDGSNR   |                    |
| ADGSGSVVLR      | CAAPTNYLFGLGDGR  |                    |
| VYDESISQLEHEGTNPCSVNNGDSCQLCLPTSETTR | CDTEDDCGDSDEPPDCPFEK |        |
| SCMCTAGYSLR     | CMDQFOCK         |                    |
| YVVISQGLDKPR    | AEGSEYQVLYIADDNEIR |                |
| AITVHPEK        | GIAIDVAGNYYWTDSGR |                |
| LYWCDAR         | IETAMGTDLR       |                    |
| TGIGVQLK        | IYWADAK          |                    |
| GTNVCAVANGGCCQQLCLYR | HSLASTDEKR |                |
| EYAGYLYSSER     |                  |                    |
|                | 18% coverage      | 22% coverage       |

*Sepp1 supplies selenium to a muscle cell line via apoER2*
Sepp1 supplies selenium to a muscle cell line via apoER2

Figure 1

Diagram showing the uptake of 75-Se in A, B, and C conditions with different time points and serum concentrations.
Figure 2

Sepp1 supplies selenium to a muscle cell line via apoER2
Sepp1 supplies selenium to a muscle cell line via apoER2

Figure 3
Figure 4

Sepp1 supplies selenium to a muscle cell line via apoER2

A

B

C

D

control siRNA > + - - + -
Lrp1 siRNA > - + - - +
apoER2 siRNA > - - + - +

relative target mRNa level (%)

$^{75}$Se uptake (cpm / $3.5 \times 10^5$ cells)
Figure 5

A

B

$^{75}$Se uptake (cpm / 3.5 x 10^5 cells)

concentration in medium (mM)
Figure 6

Sepp1 supplies selenium to a muscle cell line via apoER2
Figure 7

 apoER2 mRNA quantitation (RQ) relative to hprt

- testis
- bone marrow
- placenta
- brain
- thymus
- spleen
- quadriceps
- heart
- kidney
- liver
Long-isoform mouse selenoprotein P (Sepp1) supplies rat myoblast L8 cells with selenium via endocytosis mediated by heparin-binding properties and apolipoprotein E receptor-2 (apoER2)

Suguru Kurokawa, Kristina E. Hill, W. Hayes McDonald and Raymond F. Burk

J. Biol. Chem. published online July 2, 2012

Access the most updated version of this article at doi: 10.1074/jbc.M112.383521

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts