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Spatiotemporal expression of the *selenoprotein P* gene in postimplantational mouse embryos

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ABSTRACT Selenoprotein P (Sepp) is an extracellular glycoprotein which functions principally as a selenium (Se) transporter and antioxidant. In order to assess the spatiotemporal expression of the *Sepp* gene during mouse embryogenesis, quantitative RT-PCR and *in situ* hybridization analyses were conducted in embryos and extraembryonic tissues, including placenta. *Sepp* mRNA expression was detected in all embryos and extraembryonic tissues on embryonic days (E) 7.5 to 18.5. *Sepp* mRNA levels were high in extraembryonic tissues, as compared to embryos, on E 7.5-13.5. However, the levels were higher in embryos than in extraembryonic tissues on E 14.5-15.5, but were similar in both tissues during the subsequent periods prior to birth. According to the results of *in situ* hybridization, *Sepp* mRNA was expressed principally in the ectoplacental cone and neural ectoderm, including the neural tubes and neural folds. In whole embryos, *Sepp* mRNA was expressed abundantly in nervous tissues on E 9.5-12.5. *Sepp* mRNA was also expressed in forelimb and hindlimb buds on E 10.5-12.5. In the sectioned embryos, on E 13.5-18.5, *Sepp* mRNA was expressed persistently in the developing limbs, gastrointestinal tract, nervous tissue, lung, kidney and liver. On E 16.5-18.5, *Sepp* mRNA expression in the submandibular gland, whisker follicles, pancreas, urinary bladder and skin was apparent. In particular, *Sepp* mRNA was detected abundantly in blood cells during all the observed developmental periods. These results show that Sepp may function as a transporter of selenium, as well as an antioxidant, during embryogenesis.

KEY WORDS: *selenoprotein P, quantitative RT-PCR, in situ hybridization, mouse embryo*

Introduction

Selenium (Se) is an essential dietary trace element, which exerts important human health effects associated with immune response, cancer prevention, thyroid hormone metabolism, reproduction, and antioxidant defense functions, which are linked to these enzyme functions (Rayman, 2000). Se deficiency has been linked with cardiomyopathy, malignant tumors, deforming arthritis, immunological defects, and diseases of accelerated aging and infertility, including spermatogenesis disorders and spontaneous abortion (Rayman, 2000; Brown and Arthur, 2001).

Se exerts its biological functions via the encoding of specific *tRNA^{sec} (TrsP)* by a UGA codon into selenoprotein (Berry *et al.*, 1991). Twenty five selenoproteins in humans or 24 selenoproteins in rodents have been identified thus far, including glutathione peroxidase (GPx), thioredoxin reductase, iodothyronine deiodinase, selenoprotein P (Sepp), selenoprotein W, and other selenoproteins (Kryukov *et al.*, 2003). These selenoproteins have been shown to be capable of regulating the actions of antioxidation, redox status, thyroid hormone, glucose and Se metabolism, and sperm maturation (Stadtman, 1990; Beckett and Arthur, 2005).

Maternal Se depletion facilitates teratogenesis and death in embryos exposed to phenytoin (Ozolins *et al.*, 1996). Male mice lacking *Sepp* were infertile due to specific flagellar structural defects in the mature spermatozoa. These results also appeared to be identical to Se-deficient mice (Olson *et al.*, 2005). Targeting disruption of mouse *TrsP* induces early embryonic lethality, and the selective deletion of *TrsP* in endothelial cells, using *loxP-Cre* technology, has revealed that selenoprotein is essential to embry-

Abbreviations used in this paper: CNS, central nervous system; E, embryonic day; GPx, glutathione peroxidase; ROS, reactive oxygen species; RT-PCR, reverse transcription-polymerase chain reaction; Se, selenium; Sepp, selenoprotein P.

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TABLE 1

COMPARISON OF *SEPP* mRNA EXPRESSION IN DEVELOPING EMBRYONIC ORGANS

Organs		Embryonic day 13.5	14.5	15.5	16.5	17.5	18.5
Brain	Cerebral cortex	+++	+++	+++	+++	+++	+++
Lung	Respiratory epithelium	+++	+++	+++	+++	++	++
	Mesenchyme	++	++	++	++	++	++
Liver		+++	+++	+++	+++	++	++
Pancreas	Exocrine portion	++	++	++	+++	+++	+++
Kidney	Metanephric corpuscles	++	++	++	+++	+++	+++
	Metanephric tubules	++	++	++	++	++	++
Intestine	Surface epithelium	++	++	++	+++	+++	+++
	Muscular layer	++	++	++	+++	+++	+++
Urinary bladder	Transitional epithelium	NC	NC	NC	+++	+++	+++
	Muscular layer	NC	NC	NC	+++	++	++
Skin and hair follicle		+	++	++	+++	+++	+++
Heart		+	+	+	+	+	+
Blood cells		++++	++++	++++	++++	++++	++++

* Signal intensity:- (absence), + (mild), ++ (moderate), +++ (strong), ++++ (very strong). NC (Not checked)

onic development (Bosl *et al.*, 1997; Shrimali *et al.*, 2007).

Sepp is a Se-rich extracellular protein which harbors 50% of the total Se within the mouse plasma (Burk and Hill, 2005; Hill *et al.*, 2007). Sepp is expressed in a variety of tissues, but the greatest quantities of Sepp are generated in the liver and secreted into the plasma (Carlson *et al.*, 2004; Schweizer *et al.*, 2005). Sepp has been shown to perform crucial functions in the transportation and delivery of hepatic Se throughout the body (Hill *et al.*, 2003). *Sepp* knockout mice have been implicated in neurological dysfunctions, including motor abnormalities with severe spasticity induced by Se metabolism disorder (Hill *et al.*, 2004). The deletion of *Sepp* alters the distribution of Se to several mouse tissues, particularly the brain and testes (Hill *et al.*, 2003; Schomburg *et al.*, 2003), upregulates urinary Se excretion, and depresses whole-body Se content (Burk *et al.*, 2006; Hill *et al.*, 2007).

On the other hand, Sepp has been demonstrated to exert an antioxidant effect under both *in vivo* and *in vitro* conditions. Sepp delays oxidation to low-density lipoprotein and protects endothelial cells and human astrocytes against oxidative damage, by augmenting the expression and activity of GPx (Traulsen *et al.*, 2004; Steinbrenner *et al.*, 2006a; Steinbrenner *et al.*, 2006b).

Although it has been postulated that Sepp may be an important material for Se metabolism during embryogenesis, more comprehensive details of the roles and functions of Sepp during embryogenesis are necessary. The principal objective of the present study was to assess the relative expression levels and to identify the specific localization of *Sepp* mRNA during mouse embryogenesis for the first time. The analysis of *Sepp* mRNA expression in normal embryonic tissues might help to elucidate the specific role of Sepp.

Results

Expression level of Sepp *mRNA during postimplantational embryogenesis*

The temporal expression pattern of *Sepp* was assessed by a real-time RT-PCR analysis. As shown in Fig. 1, *Sepp* mRNA was detected in all the embryos and extraembryonic tissues on embryonic days (E) 7.5-18.5. In the embryos, *Sepp* mRNA increased gradually during embryogenesis. Interestingly, expression in the extraembryonic tissues was higher than in embryos from E 7.5 to 13.5, but decreased suddenly at E 14.5. After E 16.5, *Sepp* mRNA evidenced a similar expression level in embryos and extraembryonic tissues. *GAPDH* was utilized as an internal standard.

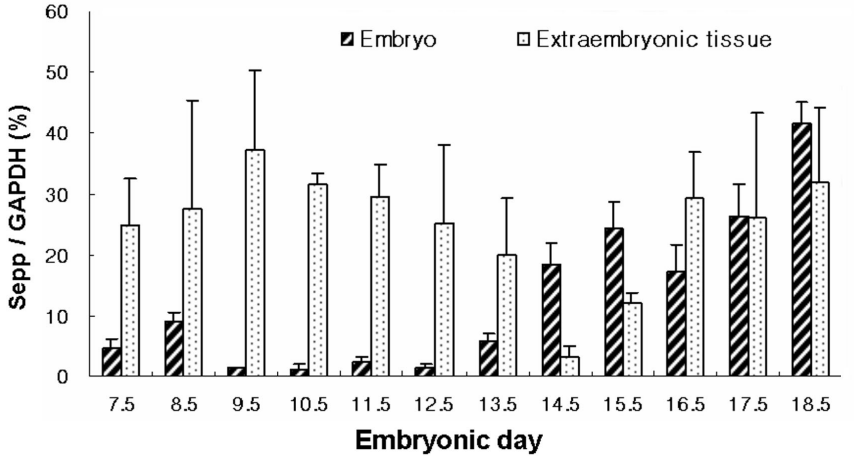


Fig. 1. Relative levels of *Sepp* mRNA expression in developing embryos and extraembryonic tissues. *Embryos and extraembryonic tissues were prepared at different developmental stages and total RNA was extracted and analyzed by quantitative RT-PCR. The expression of* Sepp *mRNA appears throughout all the embryonic stages, but shows a different pattern between embryos and extraembryonic tissues. Data represent means ± SD (n=5).*

Localization of Sepp *mRNA in whole embryos*

The spatiotemporal expression pattern of mouse *Sepp* mRNA was investigated in the embryos at E 7.5-12.5 via whole mount *in situ* hybridization. On E 7.5, *Sepp* mRNA was significantly expressed in the ectoplacental cone, trophectoderm, and decidual cells of extraembryonic tissues, but was detected weakly in the neural ectoderm of embryo (Fig. 2A). On E 8.5, *Sepp* mRNA was principally expressed in ec-

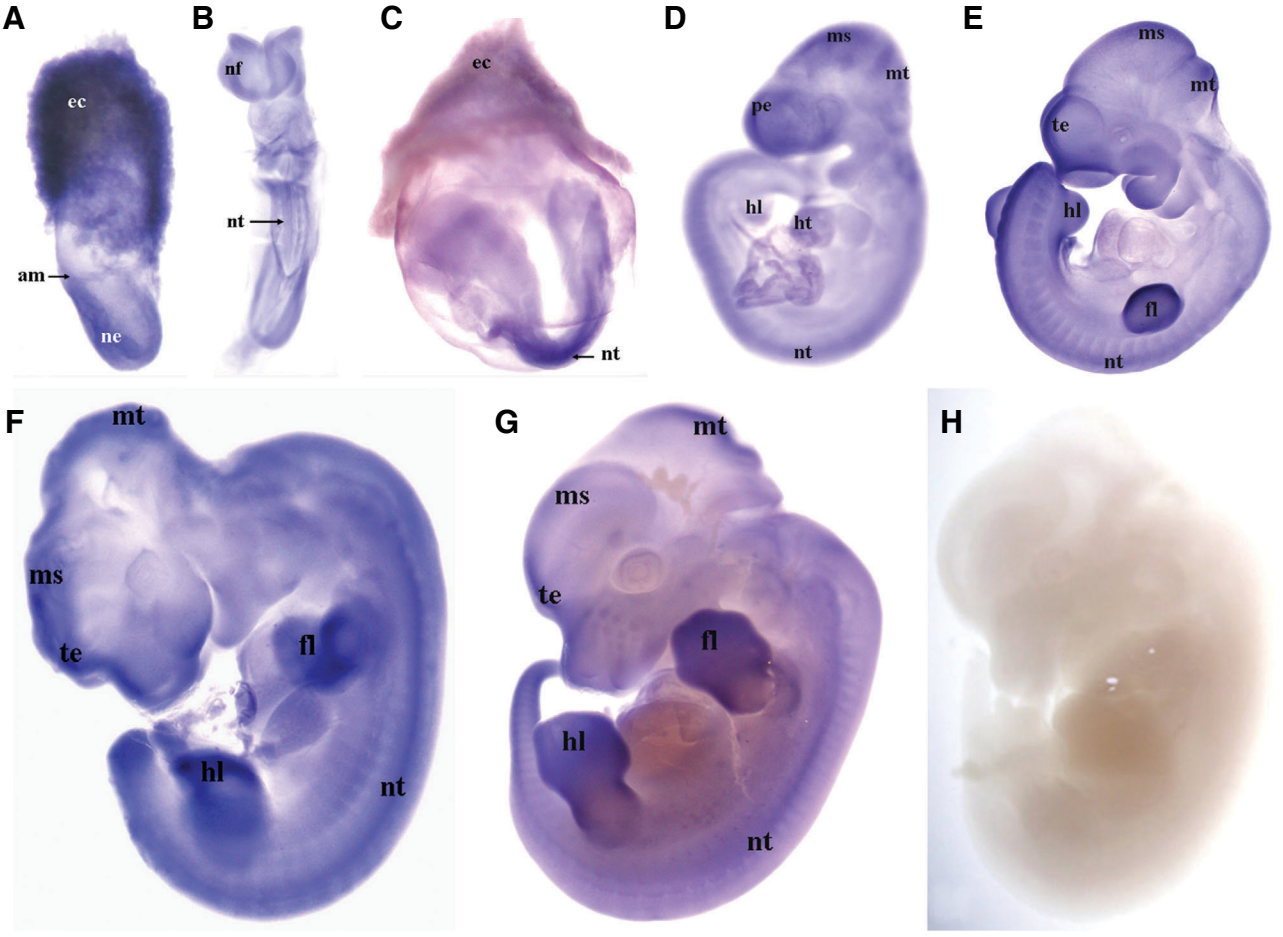


Fig. 2. Expression of *Sepp* mRNA in whole mouse embryos on embryonic days (E) 7.5 -12.5. *Embryos were hybridized with digoxigenin-labeled antisense* **(A-G)** *or sense* **(H)** *probe for* Sepp *mRNA.* **(A)** *E 7.5,* **(B,C)** *E 8.5,* **(D)** *E 9.5,* **(E)** *E 10.5,* **(F)** *E 11.5,* **(G,H)** *E 12.5. On E 7.5-8.5,* Sepp *mRNA is mainly expressed ectoplacental cone (ec), neural ectoderm (ne), neural tube (nt), and neural fold (nf). On E 9.5-12.5,* Sepp *mRNA is highly expressed in the nervous system, e.g. in the prosencephalon (pe), telencephalon (te), mesencephalon (ms), metencephalon (mt) and dorsal neural tube (nt). The signal is greatly expressed in forelimb bud (fl) and hindlimb bud (hl) after E 10.5. am, amnion; ht, heart.*

toplacental cone, neural fold, and neural tube (Fig. 2 B,C). In whole embryos after E 9.5, *Sepp* transcript was detected in the heart and central nervous system (CNS) including prosencephalon, mesencephalon, metencephalon, and dorsal neural tube (Fig. 2D). On E 10.5-12.5, *Sepp* mRNA was expressed in the developing limbs and CNS, including telencephalon, mesencephalon, metencephalon, and neural tube (Fig. 2 E,F,G). In addition, there were no apparent signals in *in situ* hybridization analysis using DIG-labeled *Sepp* sense probe (Fig. 2H).

Tissue-specific expression of Sepp *mRNA in developing embryos*

Sepp mRNA expression was assessed using tissue-sectioned *in situ* hybridization from E 13.5 to 18.5 during mouse development. In the developing nervous system, *Sepp* mRNA was expressed diffusely in the telencephalon, mesencephalon, and metencephalon, but it was primarily expressed at higher levels in the marginal zone of the cerebral cortex after E 15.5 (Fig. 3A). *Sepp* mRNA was expressed weakly in the developing hearts, whereas it was predominantly detected in the blood cells during all developmental periods (Fig. 3B). In the developing livers, *Sepp* mRNA was observed abundantly and diffusely on E 13.5-16.5, but the signal was reduced after E 17.5 (Fig. 3C). Also, *Sepp* mRNA was observed diffusely in the gastrointestinal tract during embryogenesis (Fig. 3D). In the developing lungs, the signal was detected at a higher level in bronchial epithelium than in the mesenchyme on E 13.5-16.5, but was diffusely expressed after E 17.5 (Fig. 3E). As shown in Fig. 3F, *Sepp* mRNA in metanephros was highly expressed in the metanephric corpuscles and tubules whereas it evidenced a weak expression level in the mesenchyme. *Sepp* mRNA expressions were gradually increased according to growth, and were higher in the acinar cells in the pancreas (Fig. 3G) and submandibular gland (Fig. 3H). Also, *Sepp* expression in the whisker follicles and skin were apparent on E 16.5-18.5 (Fig. 3 I,J). In the developing urinary bladder, *Sepp* mRNA was observed at a higher level in the transitional epithelium than the muscle layer after E 17.5 (Fig. 3K). In addition, the signal was detected at a higher level in the digital part of hind limb buds on E 14.5 (Fig. 3L), the Leydig cells of the testis on E 15.5 (Fig. 3M), and sensory epithelium of the inner ear on E 16.5 (Fig. 3N). There was no apparent signal in the *in situ* hybridization analysis using a DIG-labeled *Sepp* sense probe (data not shown).

The relative expression levels of *Sepp* mRNA in the developing embryonic organs were summarized in Table 1.

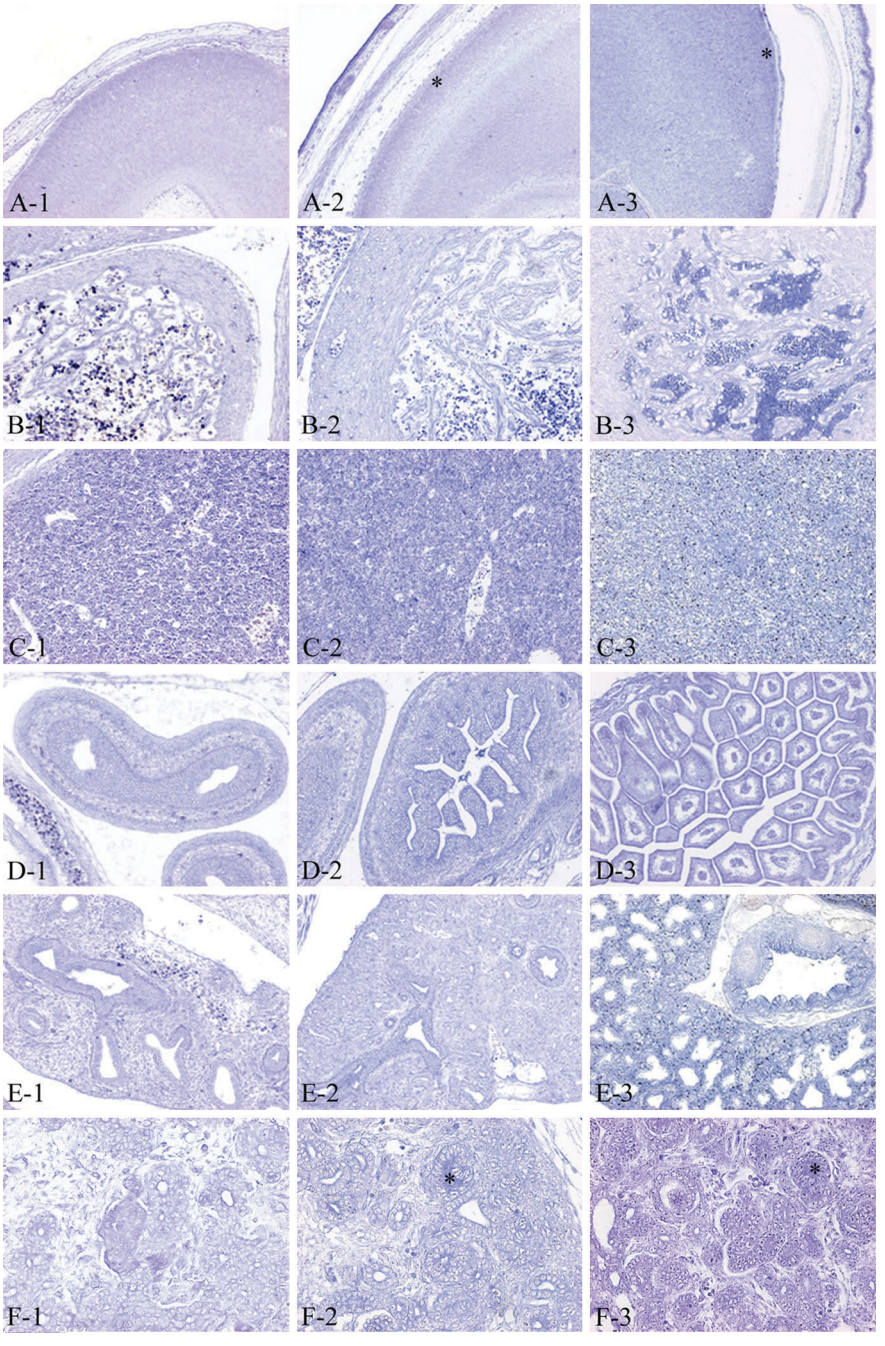
Discussion

Sepp is an abundant extracellular glycoprotein that harbors one selenocysteine in the N-terminal domain and nine other selenocysteine residues in the C-terminal domain. Sepp has two principal functions that provide Se to the various tissues via the transport of Se and function as antioxidants in the extracellular space. The C-terminal domain could be critical for the maintenance of Se in the brain and testis, whereas the N-terminal domain could be ascribed to the antioxidant function and the maintenance of Se in the kidney (Ma *et al.*, 2002; Burk *et al.*, 2003; Hill *et al.*, 2007). Se deficiency in women has been associated with infertility, spontaneous abortion, and retained placenta, thereby suggesting that Se may be necessary in mammalian embryonic development (Bedwal and Bahuguna, 1994).

During normal embryogenesis, the developing embryos generate reactive oxygen species (ROS) by utilizing both aerobic and anaerobic metabolic pathways, and require an antioxidant defense mechanism (Ornoy, 2007). Recently, we showed that antioxidant enzymes, including GPx1 and superoxide dismutase 1, were expressed throughout all mouse embryonic stages (Baek *et al.*, 2005; Yon *et al.*, 2008).

In the current study, we assessed the spatiotemporal expression pattern of *Sepp* mRNA at ontogenic stage of mouse embryo utilizing quantitative RT-PCR and *in situ* hybridization. Kasik and Rice (1995) reported that low *Sepp* mRNA expression in the mouse placenta begins to increase for 4 days before birth, and then becomes the maximum level at birth. In this real-time RT-PCR study, *Sepp* mRNA expression was detected in all embryos and extraembryonic tissues including placenta from E 7.5 to 18.5. *Sepp* mRNA was expressed abundantly in extraembryonic tissues rather than embryos on E 7.5-13.5. However, the *Sepp* mRNA level in embryos was higher than in extraembryonic tissues on E 14.5, and then appeared at a similar level in both tissues until birth. According to the results of *in situ* hybridization, *Sepp* mRNA was significantly expressed in the ectoplacental cone, trophectoderm, and the extraembryonic tissues on E 7.5-8.5. Decidual tissues originate from the maternal endometrial fibroblast and differentiate into the structure surrounding the implanting embryos. Those are generally considered to form a barrier and to provide nutrition for embryo (Bell, 1983). Collectively, these findings indicate that *Sepp* may play a role in the transplacental transport of Se from maternal fluid to the embryo in the early and middle periods of gestation, and contributes to the protection of the conceptus in late gestational periods.

In the early developmental stage, CNS begins as a simple neural plate region of the ectoderm and then folds to form the neural fold and groove. The tube is formed by the dorsal fusion of the neural folds and differentiates into the spinal cord and the brain (Roberts, 1990). In the present study, *Sepp* mRNA was expressed abundantly in the neural ectoderm, neural fold, and neural tube on E 7.5-8.5. Also, on E 9.5-18.5, *Sepp* mRNA was abundantly expressed in the CNS including the prosencephalon, telencephalon, mesencephalon, metencephalon, and spinal cord. Fantel *et al.* (1995) reported that the limb bud and brain malformation of the rat embryos were induced by ROS generation during transient uteroplacental hypoperfusion. Although no embryonic



lethality was detected in the *Sepp* knockout mice, mice with the deleted *Sepp* gene have evidenced growth defect, motor-incoordination or ataxia, and severe neurological dysfunction (Hill *et al.*, 2003; Schomburg *et al.*, 2003). These facts show that Sepp may perform a crucial role for neuronal survival, and fulfill essential functions for Se maintenance in the brain during embryogenesis.

Sepp is synthesized principally in the liver. Plasma Sepp derived from hepatocytes is the principal transport form of Se which is supported to the kidney, testis, and brain (Renko *et al.*, 2008). Accordingly, the absence of hepatic Sepp synthesis results in an increase in urinary Se excretion, which results in the depression of whole body Se concentration (Burk *et al.*, 2006). During the mid-gestational periods of mice embryos, high level of Sepp expression was observed in hepatocytes (Steinert *et al.*, 1998). In the current study, *Sepp* mRNA expression was abundant in the developing livers during E 13.5-16.5. However, the signal was decreased slightly after E 17.5. The kidney performs an important role in the maintenance of Se status and takes up Sepp from the plasma. Sepp provides Se for the biosynthesis of selenoprotein, including plasma GPx (GPx3) (Lochitch, 1989; Schweizer *et al.*, 2005). The present data indicated that the expression of *Sepp* mRNA in the developing kidney was abundantly detected in the metanephric corpuscles and tubules. Sepp is required for sperm development and is expressed predominantly in the interstitial Leydig cells in the rat and mouse testes (Koga *et al.*, 1998; Steinert *et al.*, 1998; Olson *et al.*, 2005). In the present study, *Sepp* mRNA was expressed predominantly in the interstitial Leydig cells in the developing testes. Furthermore, *Sepp* mRNA was expressed predominantly in blood cells during embryonic development. GPx activity in blood cells is tightly associated with plasma Se concentration, and Sepp also harbors the largest proportion of plasma Se (Richard *et al.*, 1991; Burk and Hill, 2005). In addition, Steinert *et al.* (1998) reported that Sepp was expressed in the hematopoietic cells clustered within the blood vessels on E 16.5. These results show that Sepp is an essential constituent in the Se-transporting pathway of developing tissues.

On the other hand, GPx3, another extracellular selenoprotein, was detected in the developing lung and intestinal epithelium. *Sepp* mRNA was also expressed within the gut epithelium on E 16.5 (Kinsley *et al.*, 1998; Steinert *et al.*, 1998). This study demonstrated a constant level of *Sepp* mRNA expression in the develop-

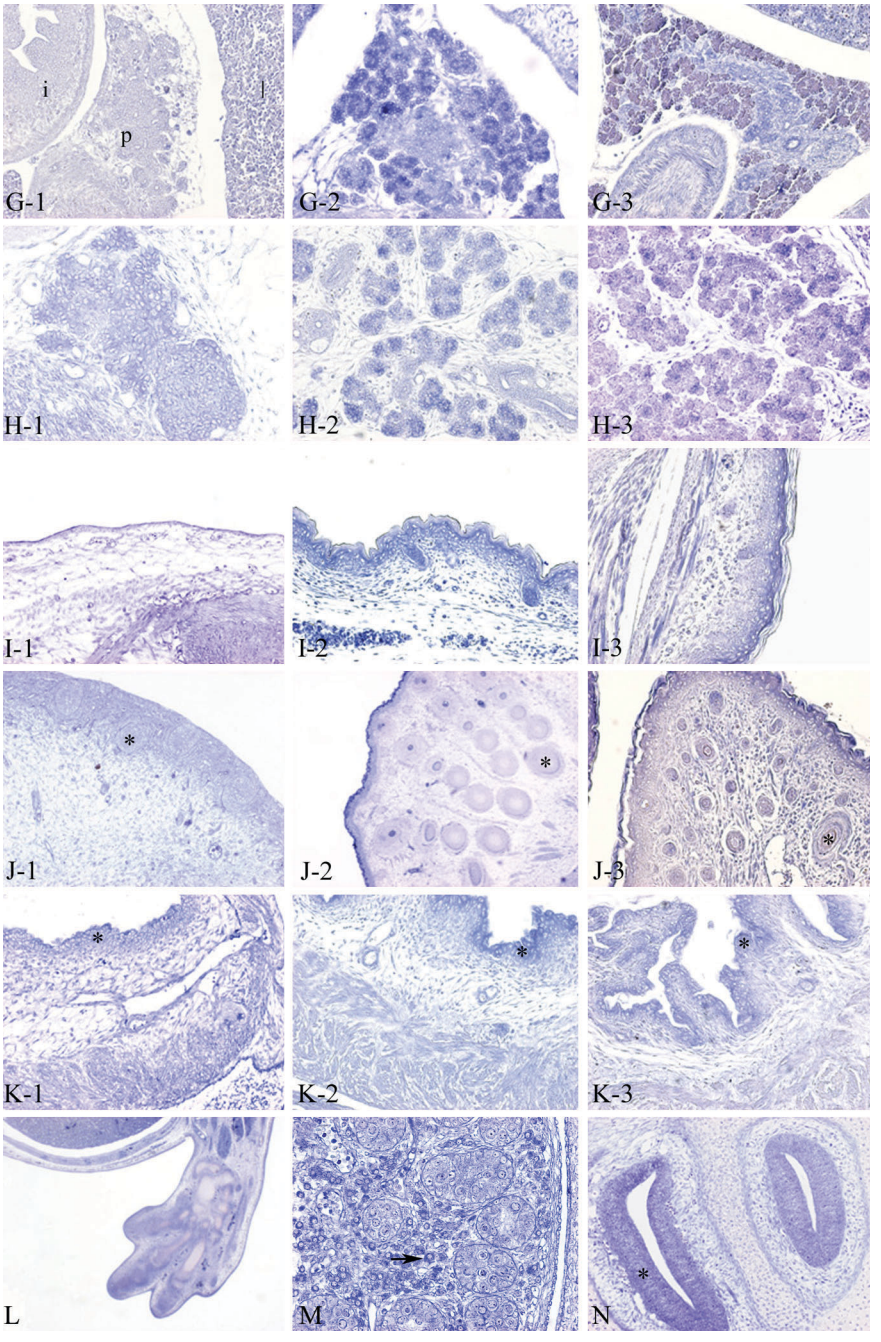


Fig. 3. Tissue-specific expression of *Sepp* mRNA in developing embryos on embryonic days (E) 13.5-18.5. *Hybridization with a* Sepp *antisense riboprobe on the sagittal sections of mouse embryos.* **(A)** *Nervous tissue; (A-1) E 14.5 metencephalon, (A-2) E 15.5 telencephalon, (A-3) E 16.5 metencephalon: marginal zone of cerebral cortex (asterisks in A).* **(B)** *Heart; (B-1) E 13.5, (B-2) E 15.5, (B-3) E 16.5: blood cells (strong signal).* **(C)** *Liver; (C-1) E 13.5, (C-2) E 15.5, (C-3) E 17.5.* **(D)** *Intestines; (D-1) E 13.5, (D-2) E 15.5, (D-3) E 18.5.* **(E)** *Lung; (E-1) E 13.5, (E-2) E 15.5, (E-3) E 17.5: bronchial epithelium (arrows).* **(F)** *Kidney; (F-1) E 13.5, (F-2) E 15.5, (F-3) E 16.5: metanephric corpuscles (asterisks in F).* **(G)** *Pancreas; (G-1) E 13.5 pancreas (p), intestine (i), and liver (l). (G-2) E 15.5, (G-3) E 17.5.* **(H)** *Submandibular gland; (H-1) E 13.5, (H-2) E 16.5, (H-3) E 17.5.* **(I)** *Skin; (I-1) E 13.5, (I-2) E 17.5, (I-3) E 18.5.* **(J)** *Whisker follicle (asterisks in J); (J-1) E 14.5, (J-2) E 16.5, (J-3) E 17.5.* **(K)** *Urinary bladder; (K-1) E 16.5, (K-2) E 17.5, (K-3) E 18.5: transitional epithelium (asterisks in K).* **(L)** *Hindlimb bud, E 14.5.* **(M)** *Testis, E 15.5: Leydig cells (arrow).* **(N)** *Inner ear, E 16.5: sensory epithelium (asterisk in N). Magnification: A (x 40), B-E, G-L & N (x 100), F&M (x 200).*

ing lung and intestine at all embryonic stages. The fetal lungs are exposed to relatively hypoxic tensions which arise suddenly at birth, and this alternation may induce oxidative injury in neonates (Araujo *et al.*, 1998). The diminution of Sepp expression in colon cancer may increase susceptibility to oxidative damage and tumor progression (Al-Taie *et al.*, 2004). As shown in Fig. 3, we distinctly noted *Sepp* expression in the whisker follicle and skin. In particular, *Sepp* transcripts were upregulated between E 16.5 and E 18.5. UV light induces oxygen radicals to exert many adverse effects in the skin. Se exerts a protective effect in UV-A damage to cultured skin fibroblasts (Leccia *et al.*, 1993). In addition, *Sepp* mRNA expression was detected at massive levels in epithelial tissues including the glandular epithelia of the pancreas and submandibular gland, transitional epithelium of urinary bladder, and the sensory epithelium of the inner ear (Fig. 3). The epithelia are specifically differentiated to perform functions for protection, absorption, secretion, excretion, and formation of a barrier for selective permeation (Dellmann and Eurell, 1998). These data show that Sepp may function as an antioxidant against excessive ROS in metabolically active sites during embryogenesis.

Sepp knockout mice lead to reduced Se content in plasma, kidney, testis and brain. Furthermore, *Sepp*- deleted male mice are infertile and *Sepp* deficiency leads to neurological impairment with ataxia and seizure (Hill *et al.*, 2003; Schomburg *et al.*, 2003; Olson *et al.*, 2005). In this study, *Sepp* gene was observed spatiotemporally in the CNS, limb buds, blood cells, lung, liver, intestine, testis, and developing epithelia, as well as extraembryonic tissues, during organogenesis. These findings indicate that Sepp may have a pivotal function to protect the embryo against oxidative damages and perform a role in transplacental and/or within the embryonic tissues in the transport of Se as a necessary material for embryogenesis.

Materials and Methods

Animals

Male and female ICR mice (8 to 10 weeks old) were purchased from a commercial breeder, Biogenomics Co. (Seoul, Korea). One male and three female mice were mated overnight in our facilities, which were maintained at 21±2ºC and 55±10% relative humidity on a 12h light/dark cycle. Pregnancy was confirmed the following morning by the presence of a vaginal plug or spermatozoa detected in the vaginal smear, which was considered as E 0.5. Under pentobarbital anesthesia, the pregnant mice were sacrificed and the embryos and extraembryonic tissues, including the placenta, were acquired from E 7.5 to 18.5. All procedures were conducted in accordance with the "Guide for the Care and Use of Animals" (Chungbuk National University Animal Care Committee, NIH # 86-23).

Quantitative RT-PCR Analysis

Total RNA was extracted from the mouse embryos and extraembryonic tissues using the Trizol reagent kit (Invitrogen, U.S.A.). Two μg of total RNA was utilized for reverse transcription (RT) to generate cDNA using a cDNA synthesis kit (Bio-Rad, U.S.A.). The generated cDNA was employed as a template for PCR reactions. Quantitative RT-PCR reactions were conducted using the TaqMan Universal PCR Master Mix Kit (Applied Biosystems, U.S.A.). *Sepp* cDNA amplification was conducted using a Model 7500 Real-Time PCR System by Assay on Demand # Mm00486049 (Applied Bioscience). The Taqman probe was FAM-labeled. Each PCR program was initiated via 2 minutes of UNG (uracil-N-glycosylase) incubation at 50ºC, followed by 10 minutes of incubation at 95ºC. Reactions were conducted in 40 cycles for 15 seconds using a denaturation temperature of 95ºC and for 1 minute with an annealing and extension temperature of 60ºC. The data were acquired and analyzed with 7500 system SDS software (version 1.3.1.21). Amplification kinetics was recorded in real-time mode as sigmoid process curves, for which the fluorescence was plotted against the number of amplification cycles. *GAPDH* mRNA was utilized as an internal standard (Assay on Demand # 4352932E, Applied Bioscience) to normalize target transcript expression. The relative ratios of *Sepp* mRNA to *GAPDH* mRNA, which can be used to quantify precisely the levels of *Sepp* expression in embryos and extraembryonic tissues, were calculated with the standard curves. The data were analyzed for duplicates of three independent runs (means±SD).

Preparation of probe and in situ *hybridization*

The spatial expression patterns were determined by whole mount *in situ* hybridization (Baek *et al.*, 2005; Yon *et al.*, 2008) using a DIG-labelled antisense probe. An antisense probe for the *in situ* hybridization was transcribed with T7 from the full length cDNA in the original pGEM-T vector digested with *Spe*I (sense: SP6). For sections, mouse embryos (E 13.5-18.5) were embedded in paraplast and sectioned. *In situ* hybridization for tissue sections was conducted as previously described protocol (Baek *et al.*, 2005; Yon *et al.*, 2008).

Acknowledgments

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References

AL-TAIE, O.H., UCEYLER, N., EUBNER, U., JAKOB, F., MORK, H., SCHEURLEN, M., BRIGELIUS-FLOHE, R., SCHOTTKER, K., ABEL, J., THALHEIMER, A., KATZENBERGER, T., ILLERT, B., MELCHER, R. and KOHRLE, J. (2004). Expression profiling and genetic alterations of the selenoproteins GI-GPx and Sepp in colorectal carcinogenesis. *Nut. Cancer* 48: 6-14.

ARAUJO, V., RUIZ, E., LLOVERA, M., TOKASHIKI, N., ABELLAN, C. and DOMINGUEZ, C. (1998). Impact of oxygen therapy on antioxidant status in newborns. Relationship with infection risk. *Biofactors* 8: 143-147.

BAEK, I.J., YON, J.M., LEE, B.J., YUN, Y.W., YU, W.J., HONG, J.T., AHN, B., KIM, Y.B., KIM, D.J., KANG, J.K. and NAM, S.Y. (2005). Expression pattern of cytosolic glutathione peroxidase (cGPx) mRNA during mouse embryogenesis. *Anat. Embryol. (Berl.)* 209: 315-321.

BECKETT,G.J. and ARTHUR, J.R. (2005). Selenium and endocrine systems. *J. Endocrinol.* 184: 455-465.

BEDWAL, R.S. and BAHUGUNA, A. (1994). Zinc, copper and selenium in reproduction. *Experientia* 50: 626-640.

BELL, S.C. (1983). Decidulaization: Regional differentiation and associated function. *Ox. Rev. Reprod. Bio.* 5: 220-271.

BERRY, M.J., BANU, L., CHEN, Y.Y., MANDEL, S.J., KLEFFER, J.D., HARNEY, J.W. and LARSEN, P.R. (1991). Recognition of UGA as a selenocysteine codon in type I deiodinase requires sequences in the 3' untranslated region. *Nature* 353: 273-276.

BOSL, M.R., TAKAKU, K., OSHIMA, M., NISHIMURA, S. and TAKETO, M.M. (1997). Early embryonic lethality caused by targeted disruption of the mouse selenocysteine tRNA gene (Trsp). *Proc. Natl. Acad. Sci. USA.* 94: 5531-5534.

BROWN, K.M. and ARTHUR, J.R. (2001). Selenium, selenoproteins and human health: a review. *Public Health Nutr.* 4: 593-599.

BURK, R.F., HILL, K.E. and MOTLEY, A.K. (2003). Selenoprotein metabolism and function: evidence for more than one function for selenoprotein P. *J. Nutr.* 133: 1517S-1520S.

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.

BURK, R.F., HILL, K.E., MOTLEY, A.K., AUSTIN, L.M. and NORSWORTHY, B.K. (2006). Deletion of selenoprotein P upregulates urinary selenium excretion and

depresses whole-body selenium content. *Biochim. Biophys. Acta* 1760: 1789 - 1793.

CARLSON, B.A., NOVOSELOV, S.V., KUMARASWAMY, E., LEE, B.J., ANVER, M.R., GLADYSHEV, V.N. and HATFIELD, D.L. (2004). Specific excision of the selenocysteine tRNA[Ser]Sec (Trsp) gene in mouse liver demonstrates an essential role of selenoproteins in liver function. *J. Biol. Chem.* 279: 8011-8017.

DELLMANN, H.D. and EURELL, A. (1998). Textbook of Veterinary Histology (5th). Philadelphia: Lippincott Williams & Wilkins. p 21.

FANTEL, A.G., PERSON, R.E., TUMBIC, R.W., NGUYEN, T.D. and MACKLER, B. (1995). Studies of mitochondria in oxidative embryotoxicity. *Teratology* 52: 190-195.

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.

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.

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.

KASIK, J.W. and RICE, E.J. (1995). Selenoprotein P expression in liver, uterus and placenta during late pregnancy. *Placenta* 16: 67-74.

KINGSLEY, P.D., WHITIN, J.C., COHEN, H.J. and PALIS, J. (1998). Developmental expression of extracellular glutathione peroxidase suggests antioxidant roles in deciduum, visceral yolk sac, and skin. *Mol. Reprod. Dev.* 49: 343-355.

KOGA, M., TANAKA, H., YOMOGIDA, K, TSUCHIDA, J., UCHIDA, K., KITAMURA, M., SAKODA, S., MATSUMIYA, K., OKUYAMA, A. and NISHIMUNE, Y. (1998). Expression of selenoprotein-P messenger ribonucleic acid in the rat testis. *Biol. Reprod.* 58: 261-265.

KRYUKOV, G.V., CASTELLANO, S., NOVOSELOV, S.V., LOBANOV, A.V., ZEHTAB, O., GUIGO, R. and GLADYSHEV, V.N. (2003). Characterization of mammalian selenoproteomes. *Science* 300: 1439-1443.

LEICCIA, M.T., RICHARD, M.J., BEANI, J.C, FAURE, H., MONJO, A.M., CADET, J., AMBLARD, P. and FAVIER, A. (1993). Protective effect of selenium and zinc on UV-A damage in human skin fibroblasts. *Photochem. Photobiol.* 58: 548-553.

LOCKITCH, G. (1989). Selenium: clinical significance and analytical concepts. *Crit. Rev. Clin. Lab. Sci.* 27: 483-541.

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.

OLSON, G.E., WINFREY, V.P., NAGDAS, S.K., HILL, K.E. and BURK, R.F. (2005). Selenoprotein P is required for mouse sperm development. *Biol. Reprod.* 73: 201-211.

ORNOY, A. (2007). Embryonic oxidative stress as a mechanism of teratogenesis with special emphasis on diabetic embryopathy. *Reprod. Toxicol.* 24: 31-41.

OZOLINS, T.R., SIKASAY, D.L. and WELLS, P.G. (1996). Modulation of embryonic glutathione peroxidase activity and phenytoin teratogenicity by dietary deprivation of selenium in CD-1 mice. *J. Pharmacol. Exp. Ther.* 277: 945-953.

RAYMAN, M.P. (2000). The importance of selenium to human health. *Lancet* 356: 233-241.

RENKO, K., WERNER, M., RENNER-MULLER, I., COOPER, T.G., YEUNG, C.H., HOLLENBACH, B., SCHARPF, M., KOHRLE, J., SCHOMBURG, L. and SCHWEIZED, U. (2008). Hepatic selenoprotein P (Sepp) expression restores selenium transport and prevents infertility and motor-incoordination in Sepp-knockout mice. *Biochem. J.* 409: 741-749.

RICHARD, M.J., ARNAUD, J., JURKOVITZ, C., HACHACHE, T., MEFTAHI, H., LAPORTE, F., FORET, M., FAVIER, A. and CORDONNIER, D. (1991). Trace elements and lipid peroxidation abnormalities in patients with chronic renal failure. *Nephron* 57: 10-15.

ROBERTS, R. (1990). The mouse: Its reproduction and development. New York: Oxford university press. p 102 & 344.

SCHOMBURG, L., SCHWEIZER, U., HOLTMANN, B., FLOHE, 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.

SCHWEIZER, U., STRECKFUSS, F., PELT, P., CARLSON, B.A., HATFIELD, D.L., KOHRLE, J. and SCHOMBURG, L. (2005). Hepatically derived selenoprotein P is a key factor for kidney but not for brain selenium supply. *Biochem. J.* 386: 221-226.

SHRIMALI, R.K., WEAVER, J.A., MILLER, G.F., STAROST, M.F., CARLSON, B.A., NOVOSELOV, S.V., KUMARASWAMY, E., GLADYSHEV, V.N. and HATFIELD, D.L. (2007). Selenoprotein expression is essential in endothelial cell development and cardiac muscle function. *Neuromuscul. Disord.* 17: 135-142.

STADTMAN, T.C. (1990). Selenium biochemistry. *Annu. Rev. Biochem.* 59: 111-127.

STEINERT, P., BACHNER, D. and FLOHE, L. (1998). Analysis of the mouse selenoprotein P gene. *Biol. Chem.* 379: 683-691.

STEINBRENNER, H., ALILI, L., BILGIC, E., SIES, H. and BRENNEISEN, P. (2006a). Involvement of selenoprotein P in protection of human astrocytes from oxidative damage. *Free Rad. Biol. Med.* 40: 1513–1523.

STEINBRENNER, H., BILGIC, E., ALILI, L., SIES, H. and BRENNEISEN, P. (2006b). Selenoprotein P protects endothelial cells from oxidative damage by stimulation of glutathione peroxidase expression and activity. *Free Radic. Res.* 40: 936-943.

TRAULSEN, H., STEINBRENNER, H., BUCHCZYK, D.P., KLOTZ, L.O. and SIES, H. (2004). Selenoprotein P protects low-density lipoprotein against oxidation. *Free Radic. Res.* 38: 123-128.

YON, J.M., BAEK, I.J., LEE, S.R., JIN, Y., KIM, M.R., NAHM, S.S., KIM, J.S., AHN, B., LEE, B.J., YUN, Y.W. and NAM, S,Y. (2008). The spatio-temporal expression pattern of cytoplasmic Cu/Zn superoxide dismutase (SOD1) mRNA during mouse embryogenesis. *J. Mol. Histol.* 39(1): 95-103.