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Review Article

Selenophosphate synthetase 1 and its role in redox homeostasis, defense and proliferation

Jiwoon Na[a,1], Jisu Jung[a,1], Jeyoung Bang[a], Qiao Lu[a], Bradley A. Carlson[b], Xiong Guo[c], Vadim N. Gladyshev[d], Jinhong Kim[a], Dolph L. Hatfield[b], Byeong Jae Lee[a,*]

[a] School of Biological Sciences, Seoul National University, Seoul 08826, Republic of Korea
[b] National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA
[c] School of Public Health, Xi'an Jiaotong University, Xi'an 710061, PR China
[d] Division of Genetics, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA

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ABSTRACT

Selenophosphate synthetase (SEPHS) synthesizes selenophosphate, the active selenium donor, using ATP and selenide as substrates. SEPHS was initially identified and isolated from bacteria and has been characterized in many eukaryotes and archaea. Two SEPHS paralogues, SEPHS1 and SEPHS2, occur in various eukaryotes, while prokaryotes and archaea have only one form of SEPHS. Between the two isoforms in eukaryotes, only SEPHS2 shows catalytic activity during selenophosphate synthesis. Although SEPHS1 does not contain any significant selenophosphate synthesis activity, it has been reported to play an essential role in regulating cellular physiology. Prokaryotic SEPHS contains a cysteine or selenocysteine (Sec) at the catalytic domain. However, in eukaryotes, SEPHS1 contains other amino acids such as Thr, Arg, Gly, or Leu at the catalytic domain, and SEPHS2 contains only a Sec. Sequence comparisons, crystal structure analyses, and ATP hydrolysis assays suggest that selenophosphate synthesis occurs in two steps. In the first step, ATP is hydrolyzed to produce ADP and gamma-phosphate. In the second step, ADP is further hydrolyzed and selenophosphate is produced using gamma-phosphate and selenide. Both SEPHS1 and SEPHS2 have ATP hydrolyzing activities, but Cys or Sec is required in the catalytic domain for the second step of reaction. The gene encoding SEPHS1 is divided by introns, and five different splice variants are produced by alternative splicing in humans. SEPHS1 mRNA is abundant in rapidly proliferating cells such as embryonic and cancer cells and its expression is induced by various stresses including oxidative stress and salinity stress. The disruption of the SEPHS1 gene in mice or *Drosophila* leads to the inhibition of cell proliferation, embryonic lethality, and morphological changes in the embryos. Targeted removal of SEPHS1 mRNA in insect, mouse, and human cells also leads to common phenotypic changes similar to those observed by in vivo gene knockout: the inhibition of cell growth/proliferation, the accumulation of hydrogen peroxide in mammals and an unidentified reactive oxygen species (ROS) in *Drosophila*, and the activation of a defense system. Hydrogen peroxide accumulation in SEPHS1-deficient cells is mainly caused by the down-regulation of genes involved in ROS scavenging, and leads to the inhibition of cell proliferation and survival. However, the mechanisms underlying SEPHS1 regulation of redox homeostasis are still not understood.

1. Introduction

Selenium is an essential trace element required in the diet of humans and other life forms. An adequate amount of selenium is essential to good health, since it is beneficial in preventing some forms of cancer, has antiviral effects, boosts the immune system, and has a role in removing reactive oxygen species (ROS) in cells. This element also has important roles in animal development and male reproduction ([1,2 and references therein)]. Most of the benefits of selenium are likely mediated by selenoproteins, which contain selenocysteine (Sec) at the active site [3–6].

Sec is the 21st amino acid in the genetic code and is inserted into the elongating peptide chain during translation by recoding the UGA stop codon to a Sec codon [7–11]. For UGA to be recoded for Sec insertion, a unique set of translation machinery composed of cis- and trans-elements is required. All selenoprotein mRNAs contain a Sec insertion

* Correspondence to: School of Biological Sciences, Interdisciplinary Program of Bioinformatics, Seoul National University, Building 504, Room 525, Seoul 151-742, Republic of Korea.
E-mail address: imbglmg@snu.ac.kr (B.J. Lee).
[1] These authors contributed equally.

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sequence (SECIS) element immediately downstream of Sec in prokaryotes and within the 3′-untranslated region (3′-UTR) in eukaryotes. The SECIS binding protein 2 (SECISBP2) binds to the SECIS element in selenoprotein mRNA. This complex in turn binds with another complex composed of the specific translation elongation factor, EFSEC, and selenocysteyl-tRNA$^{[Ser]Sec}$. This final complex, consisting of the SECIS element, SECISBP2, EFSEC, and aminoacylated Sec tRNA$^{[Ser]Sec}$, results in UGA being recoded as a Sec codon, which is then incorporated into the growing peptide chain [1,12,13].

Unlike other amino acids, which are first synthesized and then aminoacylated onto their cognate tRNA by their respective aminoacyl-tRNA synthetase, Sec is synthesized by a unique pathway following the aminoacylation of serine onto Sec-tRNA$^{[Ser]Sec}$ by seryl-tRNA synthetase [14]. The synthesis pathway of Sec is different in prokaryotes and eukaryotes. In prokaryotes, serine is initially aminoacylated onto Sec tRNA$^{[Ser]Sec}$. Sec synthase (SelA) then removes the hydroxyl group from the serine moiety of seryl-tRNA$^{[Ser]Sec}$ to form aminoacrylyl- (or dehydroalanyl-) tRNA$^{[Ser]Sec}$ that acts as an intermediate. SelA inserts selenium onto dehydroalanyl-tRNA$^{[Ser]Sec}$ using monoselenophosphate as the selenium donor. Monoselenophosphate is synthesized by selenophosphate synthetase (SelD) using selenide and ATP as substrates [15–17]. In eukaryotes, as in prokaryotes, serine is initially aminoacylated onto Sec tRNA$^{[Ser]Sec}$ by seryl-tRNA synthetase. The hydroxyl group on the serine moiety is converted to *O*-phosphoseryl-tRNA$^{[Ser]Sec}$ by phosphoseryl-tRNA kinase (PSTK). Eukaryotic Sec synthase (SecS) then utilizes *O*-phosphoseryl-tRNA$^{[Ser]Sec}$ and selenophosphate as substrates to form Sec-tRNA$^{[Ser]Sec}$ [7,18–21].

Although only one form of SEPHS (SelD) is found in prokaryotes, two isoforms of SEPHSs (SEPHS1 and 2) have been identified in eukaryotes. The functions of prokaryotic SelD and eukaryotic SEPHS2 have been established, whereas the function of eukaryotic SEPHS1 is not well understood. Nevertheless, SEPHS1 is known to have an essential role in cell proliferation and survival. SEPHS1 and SEPHS2 have a significantly high sequence homology. However, SEPHS1 does not appear to have a role in selenoprotein synthesis or function. Herein, we review the current literature on SEPHS1 and SEPHS2 to provide further insight into the function of SEPHS1.

2. The functions of prokaryotic SEPHS (SelD)

In *E. coli*, there are two forms of formate dehydrogenases, which are required for the anaerobic growth of *Escherichia coli*, formate dehydrogenase H (FDH_H) and formate dehydrogenase N (FDH_N). FDH_H converts formate to CO_2 and H_2. On the other hand, FDH_N converts formate to CO_2 by reducing nitrate to nitrite. Both contain a selenoprotein, where the selenium-containing subunit is smaller in FDH_H (80 kDa) and larger in FDH_N (110 kDa) [22,23]. Four mutants (*selA*, *selB*, *selC*, and *selD*) from *E. coli* were found and shown to prevent the incorporation of Se into FDH proteins [24]. Using complementation tests, the genes were cloned and their functions were identified: *selA*$^+$ codes for selenocysteine synthase (SecS) [15], *selB*$^+$ for EFSEC [25], *selC*$^+$ for Sec tRNA$^{[Ser]Sec}$ [26], and *selD*$^+$ for SEPHS [15]. *E. coli* SEPHS (SelD) is composed of 347 amino acids (~ 37 kDa).

Initially, SelD was found to synthesize phosphorus selenium that could serve as the selenium donor during Sec biosynthesis, but the identity of this selenium donor was not determined [27,28]. It was further shown that the phosphate moiety of phosphorus selenium originated from the gamma-phosphate group of ATP. Later this phosphorus selenium was identified as monoselenophosphate [17]. SEPHS was also purified from *Salmonella typhimurium* and was found to complement the extracts from the *selD* mutant to produce selenium donor molecules using selenide and ATP [29]. With ^{31}P NMR studies, it was found that ADP is further hydrolyzed to AMP and inorganic phosphate [29]. The cleavage of ATP by SelD is selenium-dependent and the enzyme activity of SelD is dependent on Mg^{2+} and K^+ [28,30]. As such, selenophosphate synthesis mediated by SelD can be expressed as follows:

$$ATP + selenide + H_2O \rightarrow selenophosphate + Pi + AMP$$

In this equation, the phosphate in selenophosphate and inorganic phosphate (Pi) originate from the gamma- and beta-phosphate groups of ATP, respectively. The gamma-phosphate of ATP is trapped within the enzyme after hydrolysis and forms a complex with the enzyme until it is transferred to selenide [30,31].

Interestingly, SelD is required not only for Sec biosynthesis but also for the selenylation of other tRNAs other than Sec tRNA$^{[Ser]Sec}$ [32]. The major target tRNAs for selenylation are glutamate tRNA, lysine tRNA, and glutamine $tRNA_1$ in various bacteria [27,33,34]. These tRNAs are selenylated by substituting the sulfur of 5-methyl-aminomethyl-2-thiouridine (mnm^5S^2U) with selenium to form 5-methyl-aminomethyl-2-selenouridine (mnm^5Se^2U) at the wobble position of the anticodon [32,35]. The enzyme responsible for mnm^5Se^2U synthesis (tRNA 2-selenouridine synthase, or YbbB) was isolated from *Salmonella* and *E. coli* [32,36]. In *E. coli*, the genes coding for SelD and YbbB are located at the same operon. The synthesis of mnm^5Se^2U is selenophosphate-dependent. SelD therefore plays a role as a selenophosphate supplier during the selenylation of tRNAs by mnm^5Se^2U incorporation.

3. Eukaryotic SEPHSs

A eukaryotic *selD*$^+$ homolog was cloned for the first time from the human liver cDNA library by yeast two-hybrid technology using the mammalian spliceosome-associated protein SAP62 as a bait [37]. The full-length cDNA encodes a polypeptide composed of 381 amino acids with a molecular weight of approximately 45 kDa. It has a relatively low sequence homology with *E. coli* SelD (32% identity and 55% similarity with *E. coli* SelD). However, the human SelD homolog contains a putative ATP/GTP binding motif similar to that of *E. coli* SelD. The addition of human SelD in cells harboring a 5'-deiodinase expression vector increased the levels of ^{75}Se-labeled 5'-deiodinase compared to those in the cells harboring the backbone vector, although the total radioactivity of 5'-deiodinase was very low. This SelD homolog was later renamed SEPHS1 because another SelD homolog was identified.

Another SelD homolog containing Sec at the catalytic domain was identified in eukaryotes, bacteria, and archaea [38]. Guimaraes et al. isolated mammalian SelD homologs in an effort to identify the genes that are preferentially expressed in cells that can undergo hematopoiesis at the early embryonal stage. This protein is highly homologous to SEPHS1 and consists of 448 amino acid residues, resulting in a predicted molecular weight of 47.8 kDa. Unlike SEPHS1, the gene for this protein (*Sephs2* or *Sps2*) contains an in-frame TGA codon, and ^{75}Se was incorporated into the gene product, indicating that SEPHS2 itself is a selenoprotein. The 3′-UTR of SEPHS2 mRNA was required for Sec to be incorporated into proteins during translation. The need for 3′-UTR in this gene is due to the existence of a SECIS element at the 3′-UTR of eukaryotic selenoprotein mRNAs. The SECIS element was not observed initially in the human SEPHS2 gene by *in silico* analysis owing to a low sequence homology and a different position of the SECIS elements from other SEPHS2 selenoproteins [38,39].

Subsequently, the existence of a SECIS element in SPS2 was confirmed by an experimental approach in *Drosophila* [40]. Indeed, the distance between the stop codon and the SECIS element in *Drosophila* SEPHS2 mRNA was only 30 bp, although the SECIS element is located 579 bp downstream from the stop codon in the human SEPHS2 mRNA [41]. According to Latreche et al. [42], the SECIS elements of both human and *Drosophila* SEPHS2 mRNA belong to type-2 SECIS element; furthermore, the UGA recoding ability of SEPHS2 is moderate. The main difference between type-1 and type-2 SECIS element forms is in the apical loop. Type-1 has one apical loop, whereas type-2 has two loops [42].

An *E. coli* SelD homolog with an in-frame UGA codon has also been identified in prokaryotes, including eubacteria (*Haemophilus influenza*) and archaea (*Methanococcus jannaschii*). However, there is only one form of SelD homolog in these bacteria, suggesting that the Sec incorporation system was introduced into ancestral SelD genes during the evolution of those species [38]. The finding that there are two forms of SEPHS (SEPHS1 and SEPHS2) in eukaryotes, but only one form of SEPHS in prokaryotes, raises an important question: Do both forms of eukaryotic SEPHSs have catalytic activity for selenophosphate synthesis?

There are several lines of evidence showing that SEPHS2 has the same SEPHS catalytic activity as SelD in prokaryotes. The first line of evidence was provided by an experiment performed using the *Drosophila selD*$^{+}$ homolog (*Sephs1*), expressing it in *E. coli* and assaying for ATP hydrolysis activity [43]. Unlike *E. coli* SelD, *Drosophila* SEPHS1 did not show any significant ATP hydrolysis activity to produce AMP and Pi. However, SEPHS2 produced both AMP and Pi, characteristic of SelD. Moreover, the *Drosophila* SEPHS1 gene did not complement a mutation of *E. coli selD*$^{+}$, whereas SEPHS2 did.

The second line of evidence was provided by an experiment performed in vitro to examine the ATP hydrolysis activity of mouse SEPHS1 and SEPHS2 [20]. When ATP and selenide were incubated with a mouse SEPHS2 mutant protein, where Sec is substituted by Cys, the production of selenophosphate, AMP, and Pi was detected by ^{31}P NMR spectroscopy. The reaction occurred in a selenide-dependent manner. However, the addition of mouse SEPHS1 did not result in the production of any detectable selenophosphate. Although SEPHS1 hydrolyzed ATP to ADP, further hydrolysis was limited. This SEPHS1 reaction occurred in a selenide-independent manner. It was also shown that the selenophosphate produced by SEPHS2 could be used as a direct substrate for Sec synthesis by SecS.

The third line of evidence was obtained from cell-based experiments [44]. Inducible knockdown of SEPHS2 using a small interfering RNA in NIH3T3 cells resulted in a severe reduction of selenoprotein biosynthesis. Knockdown of SEPHS1, however, did not affect biosynthesis of selenoproteins. In addition, the overexpression of mouse SEPHS2, where Sec was mutated to Cys (mSEPHS2$_{\text{Sec- > Cys}}$), the siRNA-resistant SEPHS2 (SEPHS2$_{\text{rescue}}$) construct, or *E. coli* SelD in SEPHS2-knock-down cells demonstrated the recovery of selenoprotein biosynthesis. However, overexpression of SEPHS1 in the same background cell did not increase selenoprotein levels.

The above series of experiments clearly indicate that SEPHS2 is an enzyme with the same SEPHS catalytic activity as SelD in prokaryotes. However, SEPHS1 does not contain catalytic activity for selenophosphate synthesis. As such, what is the biochemical function of SEPHS1? One interesting hypothesis is that SEPHS1 participates in the Sec recycling process presumably together with another enzyme that decomposes Sec. Tamura et al. [45] cloned human lung *SEPHS1* and *SEPHS2* genes and performed a complementation test with the *E. coli selD* mutation. While the human *SEPHS2* complemented the *selD* mutation effectively, the*SEPHS1* did not when selenite was used in the medium. The addition of Sec to the medium, however, increased SEPHS1 complementation efficiency, but the growth of wild type *E. coli* was decreased by Sec addition. Although these observations provide insight into the recycling hypothesis, more studies need to be done. Notably, the observation that SEPHS1 did not affect selenoprotein turnover in the in vivo studies [44] suggested that SEPHS1 does not increase recycling rate, even though SEPHS1 had some Sec recycling activity.

4. Evolutionary significance of two isoforms in eukaryotic SEPHSs

With the advent of next-generation sequencing technology, an enormous number of genome sequences was determined. Comparing SEPHS sequences reveals that both SEPHS1 and SEPHS2 (or SelD) contain well conserved functional domains and sequence motifs (Fig. 1). The catalytic domain is an active center for the synthesis of selenophosphate. In site-specific mutagenesis studies, it was found that Cys17 and Lys20 of *E. coli* SelD were essential for catalytic activity [46,47]. All the amino acid sequences in the catalytic domains have a high similarity across species except the amino acid residue corresponding to Cys17 of *E. coli* SelD. Prokaryotic SelD homologs and eukaryotic SEPHS2s have either Cys or Sec at the *E. coli* Cys17 position. However, SEPHS1, which has no selenophosphate synthesis activity, has a different amino acid residue, either Thr, Arg, Gly or Leu at the same position. Notably, all functional domains are well conserved.

In the ligand binding domain, Thr (designated with asterisk in Fig. 1) is known to have ADP hydrolysis activity [48]. It is conserved in all SelD homologs regardless of their selenophosphate synthesis activity. Aspartic acids in the ligand binding domain and phosphate binding domain (designated with an inverted triangle in Fig. 1) are known to form ion (Mg^{2+}) binding pockets [48,49]. Asparagine in the ligand binding domain (designated with a circle in Fig. 1) holds a gamma-phosphate group produced by ATP hydrolysis until the phosphate group forms a complex with selenide to form selenophosphate [50]. Gly and Thr of the adenosine binding domain create hydrogen bonds with the sugar and base of ADP, respectively [49]. There are an additional two motifs whose sequences are well conserved in all SelD homologs although their functions remain unclear.

Among the 215 known archaeal genomes, only 26 species (12.1%) contain a selenoprotein synthesis system, suggesting that most archaea do not utilize selenium [51]. Half of the archaea (mostly among *Methanococcus*) that utilize selenium have a Sec at the position corresponding to *E. coli* Cys17, but others have Cys at the same position. Although most eubacteria contain Cys at the *E. coli* Cys17 position, some have Sec at the same position (e.g. *Clostridium perfringens* and *Haemophilus influenza*) [52,53]. Metazoan SEPHS1 contains Thr (in vertebrates), Arg (in insects), or Gly or Leu (in tunicates) at the position corresponding to Cys17 in *E. coli* SelD.

Phylogenetic analyses provide insights into the evolution of SEPHSs. A number of independent gene duplications occurred before metazoan SEPHS1s emerged from the functional ancestor of SelD (or SEPHS2) [54, see also the Chapter 8 in ref 1]. The presence of Cys or Sec in the catalytic domain is a criterion to distinguish functional SEPHS (SelD or SEPHS2) from the non-functional counterpart (SEPHS1). In addition, the fact that all SEPHS1 and SEPHS2 are clustered separately in vertebrates suggests that SEPHS1 and SEPHS2 most likely segregated from their common ancestor prior to speciation.

Insects produce either a small number of selenoproteins (3 selenoproteins in fruit flies and mosquitos, and one in honeybees) or no selenoproteins (red flour beetles and silk worms). Interestingly, although red flour beetles and silk worms do not encode selenoprotein biosynthetic machinery, they contain the SEPHS1 gene indicating that SEPHS1 may perform a function unrelated to selenoprotein biosynthesis [55].

5. Expression, structure, and interaction of SEPHS1

5.1. Expression

The human SEPHS1 gene is composed of 10 exons. Its translational initiation and termination codons are located at exon 2 and exon 10, respectively [56]. Five splice variants were detected as shown in Fig. 2: 1) the major type (MT), 2) the variant where exon 2 is deleted (ΔE2), 3) the variant where exon 8 is deleted (ΔE8), 4) the variant containing exon 9 (+E9), and 5) the variant containing extended exon 9 (+E9α). Interestingly, there is an auxiliary initiation codon in exon 3, which can serve as an initiation codon in ΔE2 mRNA. There is another termination codon in exon 9. Therefore, the protein size of +E9 (and +E9α) is shorter than that of the MT protein, despite the mRNA of +E9 being longer than that of MT. +E9α mRNA contains a short extra oligonucleotide downstream of the termination codon in exon 9. +E9 and

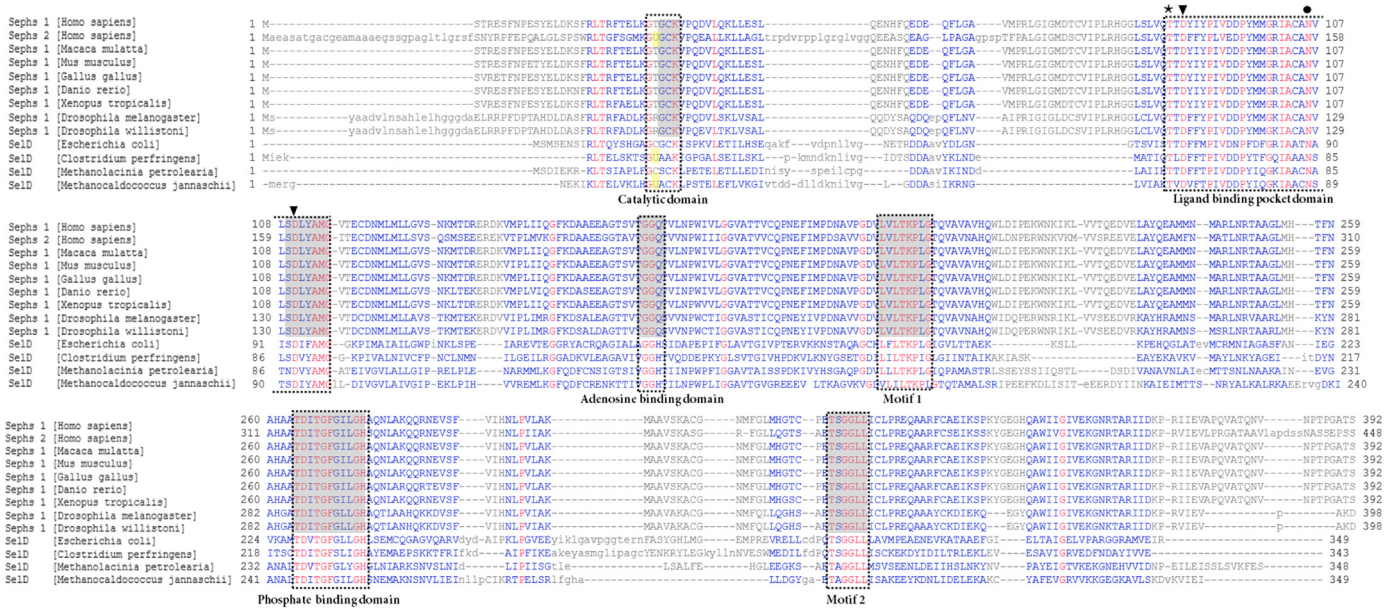

Fig. 1. Alignment of SEPHS sequences. The amino acid sequences of SEPHS from 13 representative species (9 eukaryotes, 2 eubacteria, and 2 archaea) were aligned using the NCBI COBALT program. Residues that show identity are highlighted in red, and similarities shown in blue. Lowercase letters indicate sequences present in less than 50% of the 13 species. Dotted boxes represent conserved regions, and gray boxes represent homology regions in eukaryotes. Selenocysteine residues (U) are highlighted in yellow. All SEPHS sequences used for alignment were collected from the NCBI database (https://www.ncbi.nlm.nih.gov/gene).★, ▼,and • denote conserved Thr, Asp, and Asn, respectively (see text).

+E9α produce the same polypeptides. The MT protein is the one we designated as SEPHS1. The expression pattern of these splice variants varies depending on the cell cycle stage and tissues. The MT of SEPHS1 is localized in both the nuclear and plasma membranes. However, other splice variants are located in the cytoplasm [56]. The role of each splice variant is not known. Interestingly, mammalian SEPHS2 does not have an intron [57].

SEPHS1 is expressed ubiquitously in adult human tissues [58,59]. However, the average levels of SEPHS1 mRNA are lower than those of GAPDH mRNA (approximately 1% of GAPDH). SEPHS1 expression is regulated by various factors, including intracellular selenium content, salt stress, and ROS. In the adult *D. melanogaster*, mRNA levels of SEPHS1 were higher than those of selenoproteins, including SECISBP2, when flies were grown in a chemically defined medium containing less than 10^{-7} M selenium [59]. However, the relative levels of SEPHS1 mRNA compared to those of selenoproteins/SECISBP2 decreased, when grown in a medium containing greater than 10^{-6} M selenium. Another example of the effect of selenium on *Sephs1* expression was obtained from experiments with chicken embryos [60]. As in *Drosophila*, a low concentration of selenium (10^{-9}–10^{-7} M) increased SEPHS1 mRNA levels in the central nervous system of chicken embryos. This increase in abundancy was mainly due to an increase in mRNA stability, but not due to transcriptional activation [61].

SPS1 expression was also affected by salinity in the euryhaline cichlid fish (*Oreochromis mossambicus*) [62]. Among the genes participating in selenium metabolism, the mRNAs of *Sephs1* and selenocysteine lyase (Scly) are most abundant in the gills and brain. Interestingly, the expression of SEPHS1 was significantly increased in the gills of fresh

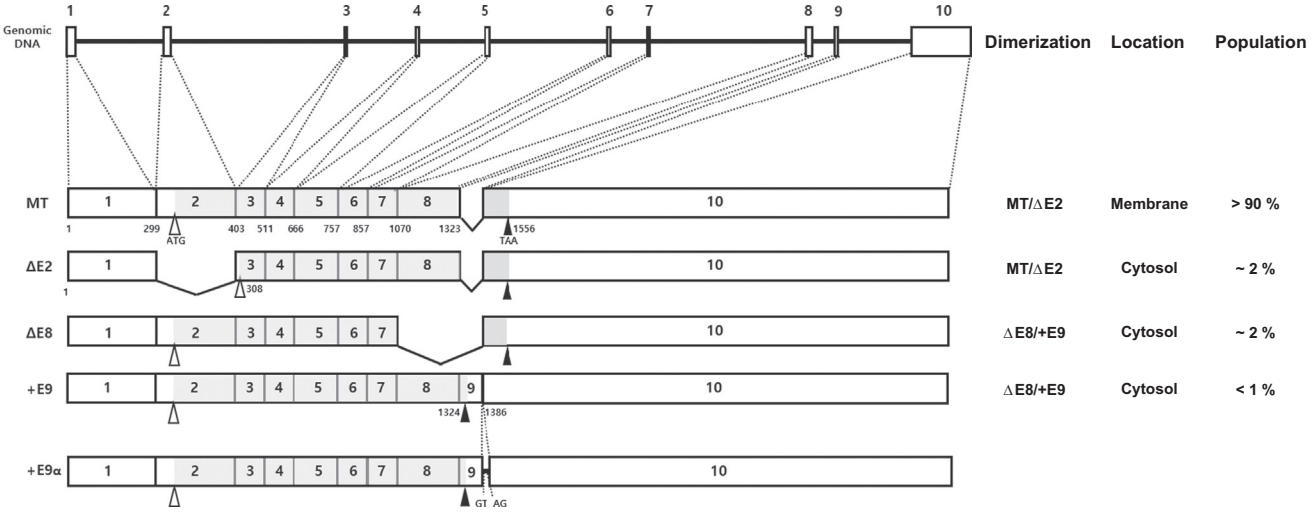

Fig. 2. The alternative splice variants of hSEPHS1 mRNA and characteristics of each variant. The black line along the top represents genomic DNA and the exons are shown as a bar. Numbers designate the exons of each predicted alternative splice variant. The non-shaded and shaded areas in the exons designate untranslated regions and open reading frames, whereas open and closed arrow heads designate the position of translational initiation and termination codons, respectively. Bent lines within cDNA designate a deleted exon in each splice variant. The numbers beneath the cDNA indicate nucleotide positions. The heterodimerization partner, location, and population of each variant are shown on the right.

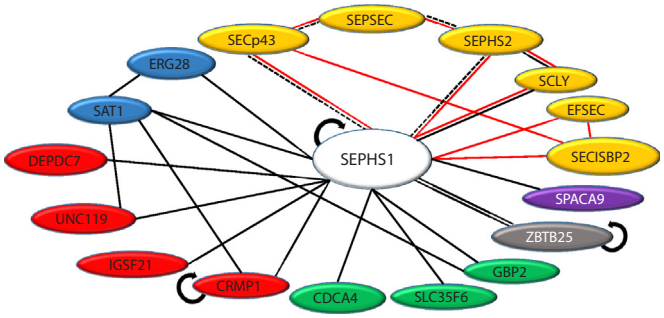

Fig. 3. The protein–protein interaction network of SEPHS1. The color of the ovoid designates the protein function in selenium metabolism (yellow), signal transduction (red), transcription regulation (gray), cell cycle regulation (green), protein binding regulation (violet), and others (blue). The black, red, and dotted lines denote the interactions identified by yeast two-hybrid, immunoprecipitation, and BRET assays, respectively. The loops designate homodimerization.

water-acclimated fish [62]. Freshwater prawns (*Macrobrachium rosenbergii*) are also euryhaline and show an inverse response to salt stress in terms of SEPHS1 expression [63]. When *M. rosenbergii* were acclimated from fresh water to salt water, *Sephs1* mRNA levels were found to increase more than three-fold compared to those of the control. These results suggest that SEPHS1 participates in the management of salt stress in euryhaline aquatic animals.

ROS levels increased after 6-hydroxyl dopamine (6-OHDA) treatment and *DJ-1* mRNA knockdown in MN9D, which is a dopaminergic neuronal cell. The elevated ROS levels led to an increase in SEPHS1 levels in the cell [64]. DJ-1 deficiency is known to be responsible for early onset of Parkinson's disease and is implicated in the protection of neurons from oxidative stress. 6-OHDA also induces ROS accumulation in neuronal cells. It is not clear whether the increase of SEPHS1 in MN9D cells with accumulated ROS is regulated at the transcriptional or post-transcriptional level. It has also been reported that an increase in ROS levels leads to p53-mediated apoptosis in neuronal cells [65]. Notably, the SEPHS1 gene has a putative p53 binding site. These findings suggest that intracellular ROS could activate *SEPHS1* transcription by the binding of p53 to its promoter region.

Fuessl et al. suggested that SEPHS1 expression has a role in lifespan and fecundity of ants [66]. For example, SEPHS1 mRNA is more abundant in the accessary gland of the winged disperser male ant (*Cardiocondyla obscurior*) than in the wingless fighter male ant. Although wingless fighter males tend to monopolize mating with young virgin queens by killing their rivals, once winged males mate with the queen, the lifespan and fecundity of the queen in *C. obscurior* is increased. This observation suggests that SEPHS1 has a function in increasing the fertility and lifespan of queen ants.

5.2. Structure

The crystal structures of SEPHS1 from humans [49], SelD from *E. coli* [51], and the SelD homolog from *Aquifex aeolicus* [50] have been determined. The overall structure of the three different types of SelD homologs, *E. coli* SelD, *A. aeolicus* SelD and human SEPHS1 are very similar. Their catalytic domains contain Cys, Sec and Thr, respectively. All three SelD homologs form homodimers with a barrel-like structure core in which each monomer contributes to the formation of one half of the barrel. Each monomer is composed of two layers. The inner layer, which forms the core, comprises the N-terminal half, while the C-terminal half surrounds the N-terminal half, which forms the outer layer.

The second and fifth beta-sheets of each monomer contribute as the dimerization interface in a two-fold symmetry related manner. In the N-terminal half, there is a narrow channel through which substrates, such as ATP and selenide, enter to the active site. The catalytic and ligand binding domains of the N-terminal half and the phosphate binding domain of the C-terminal half are located along the channel (see Fig. 1). Along this channel, there are domains such as the catalytic, ligand binding, and phosphate binding domains that hold three ATP phosphate groups. Mg^{2+} located in the channel helps ATP binding. The binding of ATP occurs through either hydrogen bonds, ionic bonds, or polar interactions. Interestingly, both the gamma-phosphate produced as a result of ATP hydrolysis and the alpha-phosphate, not beta-phosphate, of the resulting ADP form a hydrogen bond with Lys20 at the catalytic domain. While phosphate groups of ATP (or ADP) are bound to one monomer in the channel, the adenosine moiety of ATP (or ADP) interacts with the fifth beta-sheet of the counterpart monomer by forming a hydrogen bond, thereby stabilizing the dimeric structure [48].

Three-dimensional structure analyses provided important insights into the mechanism of ATP hydrolysis and selenophosphate synthesis. ATP binding to the protein stabilizes the dimeric structure and induces hydrolysis of ATP. The gamma-phosphate remains attached to the enzyme by forming various bonds with amino acid residues at the catalytic domain in the electronegative channel, such that both the gamma-phosphate and alpha-phosphate are available for further reaction [48]. Considering that the overall structures of the three proteins are similar, the main difference between the proteins is the presence of Cys (or Sec) at the catalytic domain in functional SelD and Thr at the same position in non-functional SEPHS1. Therefore, it is likely that all types of the SelD homologs commonly carry out the first step of selenophosphate synthesis as Enz + ATP - > Enz::PO_4 + ADP. However, only the functional SelD homolog containing Cys or Sec catalyzes the second step as Enz::PO_4 + Selenide - > Enz + selenophosphate in a selenide-dependent manner.

5.3. Interactions

In addition to forming a homodimer, SEPHS1 also forms heterodimers or complexes with other proteins. As shown in Fig. 3, this protein interacts with at least 17 other proteins, which has been confirmed experimentally by using various methods such as yeast two-hybrid, co-immunoprecipitation, or bioluminescence resonance energy transfer (BRET) technology [67–71]. The binding partners can be grouped based on biological processes such as in a signaling pathway (DEPDC7, CRMP1, IGSF21, and UNC119), in cell cycle regulation (CDCA4, GBP2, and SLC35F6), gene expression (ZBTB25), selenium metabolism (SEPHS2, SLA/LP, SECp43, SECISBP2, SEPSEC, and EFSEC), and other types of metabolism (ERG2 and SAT1). CRMP1 and ZBTB25 can also form a homodimer. It is not clear whether the interactions between SEPHS1 and these proteins affect their dimerization and function. It should be noted that SEPHS1 forms complexes with most proteins participating in selenoprotein biosynthesis and those proteins interact with other proteins forming a complex interaction network. For example, several different complexes composed of those proteins can be produced during selenoprotein biosynthesis such as SEPHS1-SLA/LP-Sec-tRNA$^{[Ser]Sec}$, SEPHS1-SLA/LP-Sec-tRNA$^{[Ser]Sec}$-SECp43, and SEPHS1-Sec-tRNA$^{[Ser]Sec}$-EFSEC complex [70]. Other interaction partners not thought to participate in selenoprotein biosynthesis, are implicated in cell proliferation, cell survival, cellular response to oxidative stress, and cell defense. However, the significance and mechanisms underlying these interactions regulating such cellular processes are not yet well understood.

6. SEPHS1 is required for cell proliferation

Embryonic cells and cancer cells proliferate rapidly compared to differentiated somatic cells. *Drosophila* SEPHS1 mRNA is expressed ubiquitously throughout embryogenesis and is enriched in the developing gut and in the nervous system of embryos [43,72]. Studies with *Drosophila* larvae have shown that a high level of SEPHS1 mRNA is

found in the regions where cell proliferation rate is high [73]. The abundance of SEPHS1 mRNA in the *Drosophila* embryos, especially in rapidly dividing cells, suggests that it plays an essential role in cell differentiation during embryogenesis. Indeed, knockout of*Sephs1* by P-element insertion has been shown to lead to embryonic lethality at the third instar larval/pupal stage [72]. When this gene was disrupted at the 5'-untranslated region, the imaginal disc was subject to aberrant formation. The cell number in mutant imaginal discs and in the brain was reduced, and apoptotic cells were observed in the abnormal disc. In *Sephs1* knockout in brain, the size of the brain was reduced and BrdU incorporation decreased significantly [74]. In addition, cyclin B, which is normally found in prophase/metaphase cells of wild type larval brain, was detected regardless of the cell cycle [74]. These results indicate that SEPHS1 is required for cell proliferation and survival during embryogenesis.

Knockdown of SEPHS1 mRNA using RNA interference technology in SL2 cells, a *Drosophila* cancer cell line, led to the inhibition of cell proliferation [75]. Interestingly, megamitochondria were formed by SEPHS1 deficiency in SL2 cells through the inhibition of pyridoxal phosphate (PLP, the active form of vitamin B6) synthesis. Low levels of PLP led to the increase of glutamine synthesis by activating the glutamate transporter and glutamine synthase 1. Elevated glutamine levels, presumably in mitochondria, are a sign of megamitochondrial formation [75,76]. Both megamitochondrial formation and growth retardation have been known to begin three days after double-stranded RNA is introduced into the medium, suggesting that these two phenotypes are closely correlated.

When identifying signature genes for human mature oocytes and embryonic stem cells (hESC), SEPHS1 was found as a common differentially expressed gene (DEG) in mature oocytes and hESCs compared to somatic cells [77]. This finding also supports that SEPHS1 is required for maintaining a high cell proliferation rate and that SEPHS1 can be used to establish induced pluripotent stem cells.

According to a genome-wide analysis of the mammalian transcriptome and proteome, levels of SEPHS1 are higher in cancer cells than in normal tissues from which they originated [58,59]. SEPHS1 expression is also upregulated in human rectal cancer cells. Of 12 cases (stages 2–3), 10 samples showed increased expression of SEPHS1 in cancer tissue compared to that of the normal counterpart [77]. These results support the hypothesis that a higher expression of SEPHS1 is required in cells that divide rapidly as opposed to resting cells or slowly dividing cells.

Direct evidence showing that SPS1 is essential for growth and proliferation in mammalian cells was obtained by the targeted removal of *Sephs1* or the corresponding mRNA. Systemic *Sephs1* knockout led to embryos in mice that were clearly underdeveloped by day E8.5 and virtually resorbed by day E14.5 [78]. Knockdown of *Sephs1* mRNA in both mouse and human cells also suppressed cell proliferation. Malignant properties including cell invasion and foci formation were reversed by SEPHS1 deficiency in F9 cells, a mouse embryonic cancer cell line. The effects of SEPHS1 on cell proliferation are mediated by intracellular ROS. Consistent with these observations, removal of ROS recovered cell growth and the aforementioned malignant characteristics.

7. SEPHS1 regulates redox homeostasis and defense of the cell

The first study demonstrating the effect of SEPHS1 deficiency on ROS accumulation was carried out in *Drosophila* embryos. *Sephs1* knockout in *Drosophila* by P-element insertion or depletion of SEPHS1 mRNA by RNAi led to the accumulation of ROS [73,75]. The gene responsible for this mutation was originally known as *patufet*. Subsequently, it was found to be a *SelD* homolog. Moreover, heterozygous flies with a *Sephs1* mutation exhibited a reduced lifespan when treated with oxidizing agents such as paraquat and hydrogen peroxide. However, under normal conditions, no difference in lifespan was observed [80].

Heterozygous flies that have one wild type and one null-mutant allele of *Sephs1* suppressed the rough eye phenotype induced by overexpressing the activated form of genes participating in the RAS signaling pathway (*Sev*, *Raf*, and *Rl*) [81]. Similarly, the wing vein mutant phenotypes generated by gain-of-function mutations in the Ras/MAPK signaling pathway were also rescued in *Sephs1* heterozygotes [81]. ROS are known to be used as secondary messengers for these growth factor signaling pathways [82]. Similar to the effect of *Sephs1* haploinsufficiency on Ras/MAPK signaling pathway, heterozygotes for catalase mutation ($+/Cat^{nl}$) also suppressed the mutant eye phenotype generated by hyperactivation of the Ras/MAPK signaling pathway. These observations suggest that SEPHS1 plays an important role in regulating the cellular redox balance and the accumulation of ROS by SEPHS1 deficiency or insufficiency results in the inhibition of specific growth factor signaling pathways.

As described above, the systemic knockout of *Sephs1* in mice led to lethality at the early embryonic stages. In contrast, a conditional knockout in the liver preserved viability. However, a transcriptome analysis showed that SEPHS1 deficiency in the liver significantly affected the expression of a large number of genes involved in cancer, development, and the redox regulation system [79]. Of the differentially expressed genes (DEGs), particularly notable were glutaredoxin 1 (GLRX1) and glutathione-S-transferase omega 1, whose expressions were extremely decreased in the knockout liver. The targeted removal of SEPHS1 mRNA in F9 cells showed a similar gene expression profile as in knockout liver and was found to lead to ROS accumulation, particularly in hydrogen peroxide levels, in the cells. In addition, the overexpression of GRLX1 in SEPHS1 knockdown cells led to a reversal of the elevated ROS levels, suggesting that ROS accumulation by SEPHS1 deficiency was mainly due to the inhibition in the expression of genes responsible for regulating redox homeostasis. SEPHS1 knockdown resulted in growth inhibition of F9 cells, but the overexpression of human and mouse GLRX1 led to the restoration of cell growth. These findings suggest that SEPHS1 regulates redox homeostasis, presumably by regulating the expression or activity of proteins participating in redox homeostasis.

Analysis of DEGs in SEPHS1-deficient *Drosophila* cells showed that genes participating in cell defense were activated [76]. The target genes implicated in regulating the IMD and TOLL pathways include PGRP-LF, PGRP-SD, Toll-7, attacins, cecropin, drosocin, diptericin, drosomycin, and metchnikowin. In addition, the activation of these genes is mediated by the downregulation of PLP synthesis in SEPHS1-deficient cells. A genome-wide RNAi screen using Diptercin-LacZ as a reporter also demonstrated that *Sephs1* is one of the genes that regulates the innate immune system [83].

8. Conclusion

Eukaryotic SEPHS1, which does not synthesize selenophosphate, is found in most animals, even in organisms that do not produce selenoproteins. A phylogenetic analysis of SEPHS1 and SEPHS2 sequences suggests that the amino acid residue corresponding to Cys17 of *E. coli* SelD is key for distinguishing SEPHS1 and SEPHS2 [see also ref 54]. These two isoforms most likely emerged as a result of multiple gene duplications in their common ancestors. In vertebrates, two isoforms segregated before speciation.

Generally, the SEPHS1 gene is composed of multiple exons and introns, but the SEPHS2 gene has only a single exon. In humans, *Sephs1* can produce 5 splice variants yielding four different proteins. SEPHS1 not only forms homodimers, but also binds to many other proteins including proteins participating in selenoprotein biosynthesis, cell cycle regulation, signal transduction, and transcriptional regulation. However, the biological significance of the interaction between SEPHS1 and other proteins has not yet been determined. *Sephs1* expression is affected by the intracellular levels of selenium, oxidative stress, and even salt stress. Crystal structure analysis suggests that both SEPHS1

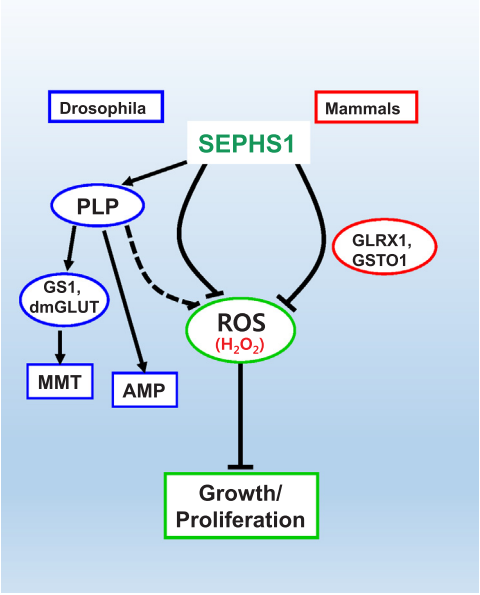

Fig. 4. A hypothetical model for SEPHS1 function. This model shows the functions of SEPHS1 in *Drosophila* and mammals. Arrows (→) designate activation, barred-lines (⊥) inhibition, and discontinuous barred-lines presumptive inhibition. PLP: pyridoxal phosphate; dmGLUT: novel type of glutamate transporter; GS1: glutamine synthase 1; MMT: megamitochondria; AMP: antimicrobial peptide; GLRX1: glutaredoxin 1; GSTO1: glutathione-S-transferase omega 1.

and SEPHS2 can hydrolyze ATP; however, the presence of Cys or Sec at the catalytic domain is key to proceed to the next step, which is selenophosphate synthesis and ADP hydrolysis.

SEPHS1 is expressed abundantly in rapidly dividing cells, such as cells undergoing development and cancer cells. SEPHS1 plays an essential role during cell development and proliferation and regulates cellular redox homeostasis and defense. The roles of SEPHS1 in cell growth and survival are likely mediated by regulating intracellular redox homeostasis (Fig. 4). In *Drosophila*, SEPHS1 regulates PLP levels, which in turn affect the formation of megamitochondria and the synthesis of antimicrobial peptides. The observation that SEPHS1 deficiency leads to the loss of malignant characteristics could provide a novel approach to the treatment of cancer. The underlying pathways and mechanisms of how SEPHS1 regulates cell proliferation and cancer malignancy are not fully understood. In addition, it remains to be shown whether SEPHS1 participates in selenium metabolism.

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