

# Chapter 10

## Mechanism, Structure, and Biological Role of Selenocysteine Lyase

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**Abstract** Selenocysteine lyase is a pyridoxal 5'-phosphate-dependent enzyme catalyzing the degradation of L-selenocysteine to L-alanine and elemental selenium. It is unique in that it acts exclusively on L-selenocysteine but not on its sulfur counterpart, L-cysteine. The enzyme is proposed to function not only in the recycling of selenium via degradation of L-selenocysteine derived from selenoproteins, but also in energy metabolism linked to obesity and metabolic syndrome. Crystallographic studies have shed light on the catalytic mechanism that allows the enzyme to distinguish between L-selenocysteine and L-cysteine, which possibly contributes in uncovering the physiological role of selenocysteine lyase in mammals.

**Keywords** Catalytic mechanism • Selenium metabolism • Selenium recycling • Selenopersulfide • Selenoprotein biosynthesis

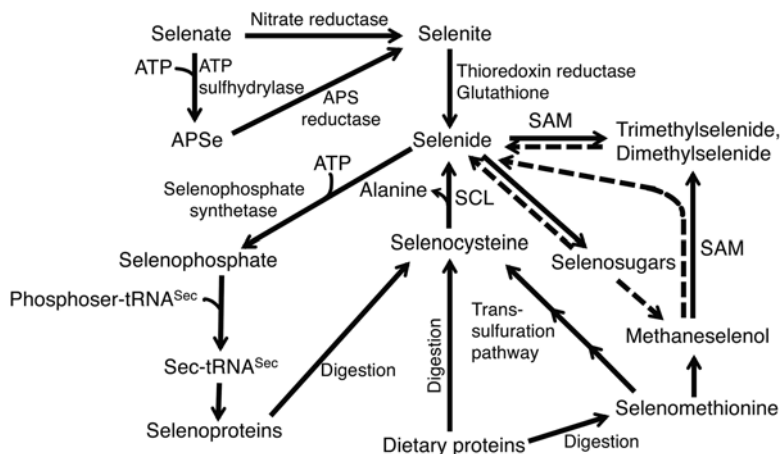
### 10.1 Mammalian Selenium Metabolism

The chemical forms of selenium in diets are mostly either selenomethionine, selenocysteine, selenite or selenate. Among these, selenomethionine is the predominant form, because about 90% of the total selenium in plants exists as protein selenomethionine residues that have randomly and frequently replaced native methionine residues [1]. Although inorganic selenium compounds are less abundant than selenomethionine, they can easily be taken up as a source of selenium for selenoprotein biosynthesis [2]. Selenate needs to be reduced to selenite prior to its utilization as a selenium source, through a mechanism similar to the nitrate reduction process. Alternatively, selenate can be reduced to selenite by adenosine 5'-phosphosulfate

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**Fig. 10.1** Selenium metabolism and incorporation of selenium into selenoproteins. *ATP* adenosine 5'-phosphate, *APS* adenosine 5'-phosphosulfate, *APSe* adenosine 5'-phosphoselenate, *SAM* S-adenosylmethionine, *SCLY* selenocysteine lyase

reductases similar to the reduction of sulfate to sulfite. Selenite is then reduced to selenide by glutathione or thioredoxin reductase.

As shown in Fig. 10.1, selenide is the key compound for selenoprotein biosynthesis. A selenocysteine residue is encoded by the UGA codon in selenoprotein mRNA, which is decoded by selenocysteyl-tRNA<sup>[Ser]Sec</sup> [3, 4]. The selenocysteine moiety on selenocysteyl-tRNA<sup>[Ser]Sec</sup> is biosynthesized using selenophosphate, which is produced from selenide and ATP by selenophosphate synthetase [5, 6]. However, the metabolic pathway providing selenide for selenophosphate synthetase remains unclear. Excess selenide is excreted in urine as trimethylselenide or dimethylselenide after methylation [7]. Selenosugar (1 $\beta$ -methylseleno-*N*-acetyl-D-galactosamine) is another excreted form of selenide that can also be utilized as a selenium source for selenoprotein biosynthesis [8, 9]. However, the bioavailability of selenosugars remains unclear, one hypothesis being that selenide or methaneselenol could be released from these.

Selenomethionine is the major dietary source of selenium for humans, and is contained in proteins from both plant and animal sources [10]. Selenocysteine can also be a selenium source from animal proteins. Free selenomethionine is easily available through the digestion of dietary proteins and is further metabolized to selenocysteine via a selenium version of the transsulfuration pathway [11]. In addition, selenomethionine is degraded by cystathionine  $\gamma$ -lyase to produce methaneselenol, which may be used as a selenium source for selenoprotein biosynthesis [12]. Methaneselenol is further methylated to dimethylselenide and trimethylselenide, which might also be utilized as a selenium source after demethylation [10]. However, little is known about the chemistry of their demethylation. Selenocysteine is also produced through digestion of internal or external (namely dietary) proteins, whereas free selenocysteine is rarely found in cells according to speciation studies [13]. Nevertheless, free L-selenocysteine is the specific substrate of selenocysteine

lyase (SCLY), which decomposes selenocysteine into selenide and alanine [14]. Subsequently, selenium can be recycled via SCLY for the synthesis of new selenoproteins (Fig. 10.1). The free form of selenide is less commonly present in organisms than the protein-bound form [15]. Selenium is liberated from selenocysteine by SCLY and covalently bound to the particular cysteine residue of the enzyme as described below. Therefore, SCLY itself is regarded as one of such selenium-binding proteins.

## 10.2 Identification of SCLY in Mammals and Bacteria

A study on selenocysteine biosynthesis in rat liver homogenates [11] revealed the existence of SCLY (EC 4.4.1.16) catalyzing the degradation of selenocysteine to form alanine and selenide. SCLY was then purified to homogeneity from pig liver and characterized [14]. The enzyme from pig is a homodimer with a subunit  $M_r$  of 48,000, each containing one molecule of pyridoxal 5'-phosphate (PLP) as a coenzyme. It shows a typical PLP-enzyme, UV-visible spectrum with maximum absorption at 420 nm. L-Selenocysteine is stoichiometrically converted to  $H_2Se$  and L-alanine in the presence of excess dithiothreitol (DTT), with a specific activity of 37  $\mu\text{mol}/\text{min}/\text{mg}$  at an optimal pH of 9.0. On the other hand, if DTT is not added in excess to the reaction mixture, an elemental form of selenium,  $Se^0$ , is formed from L-selenocysteine generating a red precipitate. Thus, the intrinsic product of the enzymatic reaction is  $Se^0$  and not  $H_2Se$  as  $Se^0$  is spontaneously reduced to  $H_2Se$  when excess DTT is added to the standard reaction mixture. The  $K_m$  value for L-selenocysteine is 0.83 mM, which is comparable to the  $K_i$  for L-cysteine, a competitive inhibitor of SCLY. The cellular concentration of free L-selenocysteine is probably much lower than the  $K_m$  value whereas free L-cysteine is generally found at concentrations similar to the  $K_i$  value. Therefore, the cellular SCLY reaction probably happens extremely slowly, suggesting that an unknown mechanism might exist to specifically deliver L-selenocysteine to SCLY in cells.

SCLY activity occurs in the homogenates of various mammalian tissues such as liver, kidney, pancreas, adrenal gland, heart, lung, testis, brain, thymus, spleen, and muscles of rat, dog, mouse, pig, and other mammals [14]. Liver and kidney homogenates showed particularly high activities compared to others. This conforms with the results of Western blot analysis of proteins extracted from mouse tissues in which Scly was abundantly present in the liver, kidney, and testis [16]. On the contrary, little Scly activity was found in blood and fat tissues.

The first cDNA cloning of mammalian Scly was reported in 2000 for the mouse Scly gene [16]. The cDNA for mouse Scly is 2172 bp in length, with an open reading frame encoding a polypeptide chain of 432 amino acid residues ( $M_r=47,201$ ). The recombinant mouse SCLY overproduced in *Escherichia coli* is a homodimer with a subunit  $M_r$  of 47,000. cDNA cloning has also been performed for the rat gene [17]. Steady-state kinetic analysis of the recombinant rat SCLY revealed that the  $V_{\text{max}}$  and  $K_m$  values for L-selenocysteine were 26  $\mu\text{mol}/\text{min}/\text{mg}$  and 5.5 mM, respectively.

Prokaryotes also have a similar enzyme, which was purified from *Citrobacter freundii* [18, 19]. The bacterial enzyme is markedly different from the mammalian enzyme with respect to its physicochemical properties and amino acid composition. Contrary to the homodimeric pig liver SLCY enzyme with a subunit  $M_r$  of 48,000, the bacterial enzyme is monomeric with a  $M_r$  of 64,000 [19]. Nevertheless, the enzyme is very similar to the mammalian enzyme regarding its enzymatic properties, that is, catalyzing the degradation of L-selenocysteine into L-alanine and selenium, but being inert against L-cysteine. The apparent  $K_m$  for L-selenocysteine is 0.95 mM, and the enzyme shows maximal activity at pH 7.0. L-Cysteine behaves as a competitive inhibitor of the enzyme with a  $K_i$  of 0.65 mM. SCLY activity is also found in some bacterial strains such as *Alcaligenes viscolactis* and *Pseudomonas alkanoalytica* [18]. However, none of the bacterial *Scly* genes have been identified or cloned to date, and their biological roles remain unknown.

### 10.3 Orthologous Genes in Various Organisms

A number of genes sharing sequence homology with *Scly* have been deposited in the nucleotide sequence databases. These include orthologous genes in all vertebrate genomes that have been sequenced to date, such as in mammals (human, chimpanzee, rhesus monkey, rat, dog, giant panda, cow, pig, horse, opossum, and platypus), birds (chicken and zebra finch), amphibians (African clawed frog and Western clawed frog), and fishes (zebrafish, Japanese puffer fish, and green spotted puffer). Interestingly, the unicellular choanoflagellate, *Monosiga brevicollis*, which is among the closest unicellular relatives of animals, also has a gene with moderate (>36%) sequence similarity with *SCLY*. Apart from *NifS*-type genes (described below), no other genes significantly homologous to *Scly* were found in the genomic sequences of plants, fungi, bacteria, and archaea, suggesting that SCLY activities detected in some bacterial and archaeal strains are probably related to side reactions generated by *NifS*-type cysteine desulfurases (CDS) and/or catalytic actions provoked by unidentified monomeric bacterial SCLY, significantly different from mammalian SCLY.

### 10.4 Homology to *NifS*-type Cysteine Desulfurases

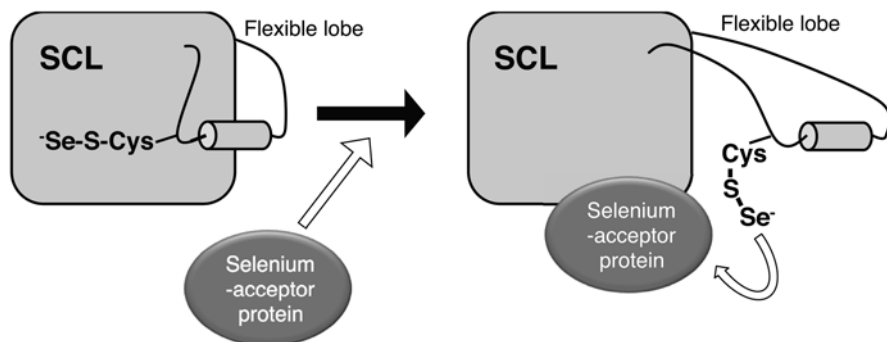
Mammalian SCLY is distantly related to *NifS*-type CDS in the primary structure with overall sequence identity of less than 30% [16, 20]. CDS catalyzes the desulfurization of L-cysteine to provide sulfur for iron-sulfur clusters, thiamine, molybdopterin, and thionucleotides in tRNA [21]. The CDS enzymes family generally uses both cysteine and selenocysteine as substrates, but with different catalytic rates [22–26]. However, the physiological significance of selenocysteine degradation by CDS has not been clarified. Unidentified genes with weak similarity to both *SCLY*

and CDS genes have been detected in the genomes of diatoms, green algae, and euglenozoans such as *Trypanosoma brucei* and *Leishmania infantum* [20]. However, their catalytic actions on selenocysteine and cysteine remain unknown.

## 10.5 Structure and Open-Close Conformational Change

Crystal structures of SCLYs from rats [17] and humans [27] have been solved with accession numbers 3A9X (rat Scly (rScly) in a native form), 3A9Y (rScly in complex with L-cysteine), 3A9Z (rScly in complex with selenopropionate), 3GZC (human SCLY (hSCLY) in a native form), and 3GZD (hSCLY in another native form). The protein folds of both SCLYs are similar to those of *E. coli* IscS [28], *Thermotoga maritima* NifS-like protein [29], *Synechocystis* sp. PCC 6803 SufS [30], *E. coli* CsdB/SufS [31–33], *Synechocystis* cysteine C-S lyase [34], and *Pseudomonas fluorescens* kynureninase [35]. In fact, they all belong to the same Fold type I family of PLP-dependent enzymes [36, 37]. CDSs are known to be divided into two major families: Group I, including IscS and NifS-like proteins, and Group II, including SufS and CsdB proteins [21, 23, 38]. SCLY shows higher similarity with Group I CDS than with the others [16].

SCLY has an important cysteine residue, Cys375 of rScly, which is completely conserved among mammalian SCLYs. Cys375 of rScly lies within a flexible extended lobe (Ser374-Ile392) and is located near the cofactor PLP, forming a Schiff base with Lys247 at the active site [17]. A mutant SCLY (C375A), in which Cys375 is replaced by alanine, exhibits no activity on selenocysteine, suggesting that Cys375 is essential for the catalytic process. Electrospray ionization-mass spectrometry of rScly incubated with L-selenocysteine revealed the binding of one selenium atom to Cys375 per molecule: a cysteine selenopersulfide intermediate (Scly-S-Se<sup>-</sup>) was formed at Cys375 [17]. Note that no such molecular species appeared when the C375A mutant enzyme was used. The X-ray structure of the rScly•selenopropionate complex indicated that the thiol group of Cys375 interacts with the selenolate part of selenopropionate, although it is depleted of the amino group and differs from selenocysteine. Therefore, Cys375 probably plays an important role in catalysis of bringing selenocysteine to a key position that facilitates the formation of a Schiff base with PLP. Moreover, the small domain (denoted by the flexible lobe in Fig. 10.2) moves towards the active site, which enables the creation of an ordered structure (otherwise disordered in the unliganded form) allowing the encapsulation of selenopropionate within the active site cavity [17]. This conformational change was also observed in the X-ray structure of the rScly•L-cysteine complexes. This open-closed conformational change enables the active site to be completely covered (as if protected with a lid) and protect the bound selenium from the solvent. The disorder-order transition of the flexible lobe probably plays a pivotal role in the catalytic event, as Cys375 is then oriented properly for the correct interaction with the selenol of the substrate. In addition, the oxygen-sensitive selenopersulfide formed in the cavity is effectively shielded from the solvent. A similar open-closed conformational change was reported in the crystal structure of hSCLY [27].

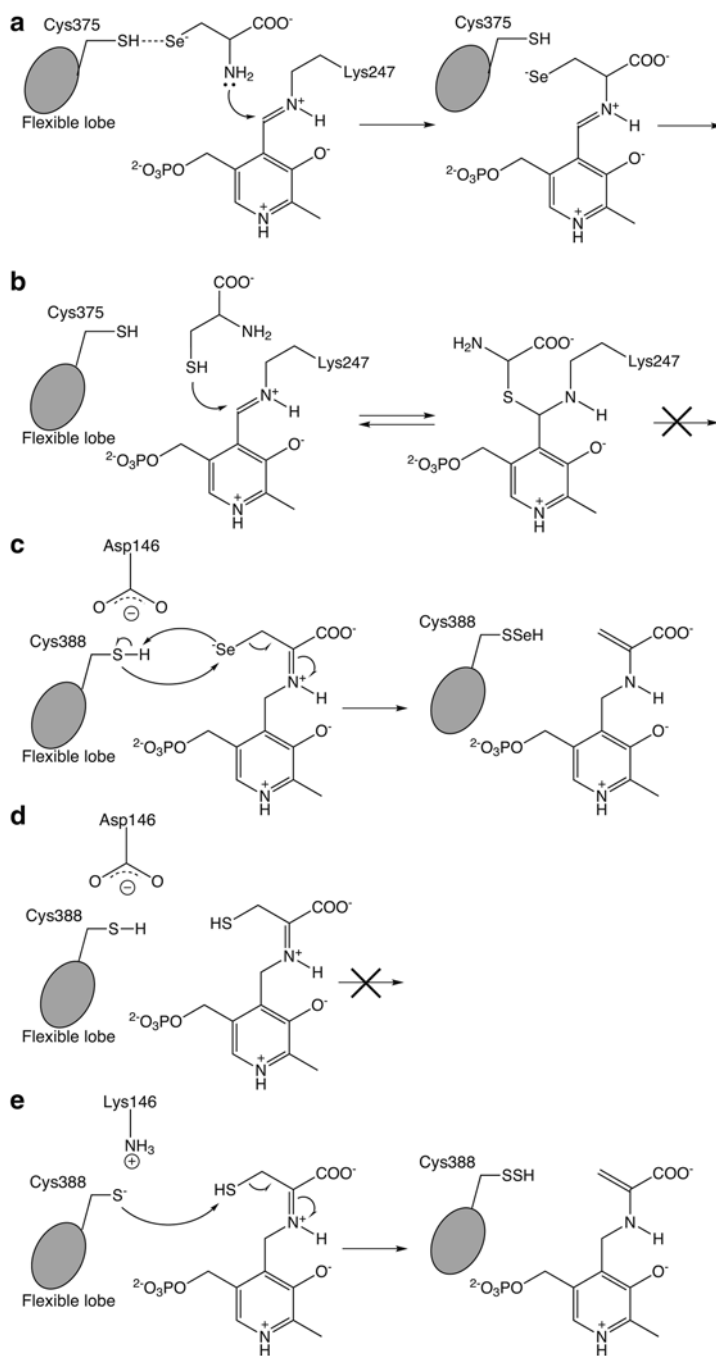


**Fig. 10.2** Possible mechanism of selenium delivery to a selenium-acceptor protein. Selenopersulfide formed on the active site cysteine residue (corresponding to Cys375 of rat SCLY) is located on the flexible lobe. Selenopersulfide selenium may be directly transferred to a specific selenium-acceptor protein via a conformational change of the flexible lobe associated with protein-protein interaction

## 10.6 Catalytic Mechanism

The reaction mechanism of SCLY has been independently studied by two groups [17, 27, 39]. The models presented for rSclY [17] and hSCLY [27, 39] are similar except for a slight difference. The small domain rotation and the ordering of the flexible lobe occur with the binding of selenocysteine at the active site of SCLY as described above. Thereby, its  $\gamma$ -Se atom can interact with the thiol group of Cys375 (Fig. 10.3a). The amino group of L-selenocysteine should be deprotonated in order to form the external aldimine with PLP, thus enabling the release of the  $\epsilon$ -amino group of Lys247 in a deprotonated form. This form is essential to act as a base to allow the removal of the  $\alpha$ -hydrogen from the substrate L-selenocysteine. The protonated Lys247 that is formed can then provide a proton to the C4' of the quinonoid intermediate, generating the substrate-ketimine intermediate. The substrate-ketimine subsequently transfers selenium to Cys375 to form cysteine selenopersulfide (Cys375-S-Se<sup>-</sup>) and the alanine-enamine intermediate, which is eventually converted to alanine, while bringing the enzyme back to its initial state. It remains unclear whether selenium is directly released from Cys375-S-Se<sup>-</sup> or is trapped by a selenium-transferring protein and subsequently released by a reductant in the

**Fig. 10.3** (continued) resulting in the production of the external aldimine to initiate the catalytic reaction. (b) When L-cysteine enters the active site, the S $\gamma$  atom of the bound cysteine, unlike the  $\alpha$  amino group, creates a covalent bond with the C4' atom of PLP in order to form a reversible non-productive adduct. (c) Protonated Cys388, which is stabilized by Asp146, is able to eliminate selenium from the selenocysteine-ketimine intermediate. (d) Protonated Cys388 cannot interact with the protonated S $\gamma$  atom of the cysteine-ketimine intermediate, resulting in the termination of the reaction. (e) When Asp146 is replaced by Lys, the deprotonated Cys388, which is stabilized by Lys146, can eliminate the S $\gamma$  atom of the cysteine-ketimine intermediate



**Fig. 10.3** Proposed mechanisms for the discrimination between selenocysteine and cysteine by rat SCLY (**a** and **b**) and human SCLY (**c**–**e**). (**a**) Interaction between the active site Cys375 (in protonated form) and the substrate selenolate allows selenocysteine to form the Schiff base with PLP,

reaction system. If selenopersulfide selenium, which is more sensitive to oxygen than persulfide sulfur, is released directly from an intermediate and diluted by the bulk solvent, its delivery to a specific acceptor molecule would be inefficient. The selenium atom of the selenopersulfide is probably transferred to a selenium-acceptor protein yet unidentified, presumably via specific protein-protein interactions. Selenophosphate synthetase is one of the most promising candidates for such a selenium acceptor role. The following studies support this thought: CDSs from *E. coli* [40] and *Methanococcus vannielii* [41] as well as mouse SCLY (Mihara et al., unpublished results) effectively provided selenophosphate synthetase with selenium from L-selenocysteine in order to produce selenophosphate. Another study using co-immunoprecipitation with a reticulocyte lysate system also indicated that SCLY associates with either selenophosphate synthetase isozymes, SEPHS1 and SEPHS2 [42]. Further studies with a yeast 2-hybrid screening system have shown that SCLY interacts with various proteins from mouse cDNA libraries related to spermatogenesis, protein synthesis, cell viability, and apoptosis as well as major urinary proteins [42]. These studies may provide clues to identify selenium-acceptor proteins from SCLY and to understand their physiological significance.

## 10.7 Discrimination Between Selenium and Sulfur

Sulfur and selenium atoms resemble each other, and most enzymes acting on sulfur compounds cannot distinguish between these atoms within substrate molecules [11, 43]. However, SCLY is markedly different from CDSs because the latter act indiscriminately on both selenocysteine and cysteine. The strict specificity of SCLY towards selenocysteine can be understood carrying out ultraviolet-visible spectral and crystallographic studies of the C375A mutant of rSCLY [17]. While inactive, the C375A mutant shows the same spectral change when forming a complex with either L-selenocysteine or L-cysteine. In the absence of Cys375, L-selenocysteine cannot form a Schiff base with PLP, and its amino group interacts with the 3'-OH group of PLP. The nitrogen atom of the lysyl residue forming a Schiff base is then deprotonated as in the wild type enzyme complexed with L-cysteine (Fig. 10.3b). Cysteine is distinct from selenocysteine because its thiol group is mostly in a protonated form due to its higher  $pK_a$  value (around 8.5). Consequently, the thiol group of L-cysteine interacts with the nitrogen atom of the lysyl residue, forming a Schiff base and thus generating a non-productive complex (Fig. 10.3b). On the other hand, when the wild type SCLY forms a complex with L-selenocysteine (Fig. 10.3a), the thiol group of Cys375 not only acts as the acceptor for the selenium atom liberated from selenocysteine, but also interacts with the deprotonated selenol of selenocysteine (Fig. 10.3a). Thus, the amino group of L-selenocysteine is free to react with the C4'-carbon of the internal Schiff base, leading to the formation of an external aldimine, an essential productive complex as described in Sect. 10.6. A similar mechanism for the discrimination between selenocysteine and cysteine is proposed from the analysis of hSCLY: the protonated form of the active site Cys388 (corresponding



to Cys375 of rScly) is a key factor in conferring specificity of the enzyme towards selenocysteine [39] (Fig. 10.3c–e). Asp146 of hSCLY, which is conserved among all known mammalian SCLYs, is thought to stabilize the deprotonated state of the active site, Cys388, because conversion of Asp146 to lysine, a common feature among CDSs, generated hSCLY with CDS activity [27, 39].

## 10.8 Biological Role

Selenoprotein P (Sepp1) is unique in that it has multiple selenocysteine residues in a unique two-domain structure. The N-terminal domain only displays one selenocysteine while the C-terminal domain contains many selenocysteine residues, nine in human SEPP1, for example [10]. SEPP1 is the major plasma selenoprotein and is predominantly synthesized in liver before being transported to various organs by the lipoprotein receptor-related proteins megalin and ApoER2 (see Chap. 22). SEPP1 retains local selenium by preventing renal excretion and also acts as a reversible storage device for selenium in brain. Therefore, the deletion of the *Sepp1* gene in mice causes severe neuronal abnormality [44]. Gene silencing studies showed that SCLY is required for the utilization of selenium derived from SEPP1 during selenoprotein biosynthesis in HeLa cells [45]. Nevertheless, *Scly*-knockout mice showed no apparent behavioral or phenotype changes, unlike *Sepp1*-knockout mice [46]. Therefore, SCLY can be readily replaced by other enzymes such as NifS-like CDSs, even though present in normal selenium metabolism. However, *Scly*-knockout mice fed with low-selenium diet displayed a subtle learning deficit and showed a significantly reduced expression of selenoproteins in brain [46]. Moreover, the simultaneous deletion of *Sepp1* and *Scly* genes in mice generated a worsened phenotype compared with single *Sepp1*-knockout mice [47]. Double-knockout mice needed supraphysiological selenium supplementation to survive, even though this addition does not cure the neuronal abnormality. In addition, selenoprotein levels in the brain are reduced in double knockout mice [47]. Therefore, SCLY must play a hitherto unknown role in supporting SEPP1 metabolism, which cannot be fully compensated by other enzymes such as NifS-like CDSs, to maintain selenium homeostasis in brain.

SEPP1 also plays a role in testis and kidney, where SCLY is highly expressed [10]. SEPP1 is transported through the receptor ApoER2, which is abundantly found in Sertoli cells [48]. However, SCLY is not expressed in these cells, and it is not clear whether SCLY has functions as a support for SEPP1 metabolism in testis. Furthermore, it is evident that SCLY is not only present in nuclei of cultured HeLa cells, but also resides in nuclei of mouse organs such as kidney and testis even though the enzyme does not display any clear nuclear localizing signals [45]. The components of the selenoprotein biosynthesis machinery such as tRNA<sup>[Ser]Sec</sup>, SEPHS2, and SECISBP2 are known to be found in the nucleus. In addition, mature selenocysteyl-tRNA<sup>[Ser]Sec</sup> is protected against nucleocytoplasmic shuttling [49]. Selenoprotein mRNAs are also prevented from nonsense-mediated decay through assemblage with the selenocysteine incorporation complex [50]. Therefore, it may

be reasonable to suggest that SCLY also participates in the selenoprotein biosynthesis machinery complex if it acts in selenium metabolism.

Recent studies have shown that *Scly*-knockout in mice causes diet-induced obesity and enhances the development of metabolic syndrome as compared to wild type mice, even with adequate dietary selenium intake [51, 52]. Interestingly, only the expression of serum SEPP1 was increased by the knockout of *Scly* [52]. Obesity may be due to increased expression of SEPP1, although further studies are required to clarify the physiological significance of this phenomenon. Furthermore, *Scly*-knockout mice showed higher pyruvate levels in liver, higher expression of pyruvate carboxylase and pyruvate dehydrogenase, and increased activity of citrate synthase. These features are all related to the tricarboxylic acid cycle and fatty acid biosynthesis [52]. The yeast two-hybrid system studies showed interactions of SCLY with various proteins related to the energy metabolism such as NADH dehydrogenase [53], as well as other enzymes participating in carbohydrate and lipid metabolism such as aldehyde reductase [42]. Thus, SCLY may have some interesting, but yet unknown, functions in energy metabolism. In the future, further studies will be required to shed light on the biological functions of SCLY.

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