Chapter 9
Structure and Mechanism of Selenocysteine Synthases

Malgorzata Dobosz-Bartoszek and Miljan Simonović

Abstract Selenocysteine synthases, SelA in bacteria and SepSecS in archaea and eukaryotes, catalyze the terminal reaction of selenocysteine synthesis during which either serine or phosphoserine is converted into selenocysteine while being attached to $tRNA^{Sec}$. The reaction mechanism is based on the PLP co-factor and requires selenophosphate as the selenium donor. Although adopting different structures, SelA and SepSecS employ similar general principles for substrate recognition and chemical catalysis. Five SelA homodimers assemble into a ring structure that harbors ten tRNA-binding and catalytic sites. Each SelA subunit binds to the D-arm and T loop of $tRNA^{Sec}$. By contrast, two SepSecS homodimers arrange into a tetramer that binds to the acceptor and variable arms of $tRNA^{Sec}$. In both instances, at least two enzyme homodimers are needed to bind and act on one $tRNA^{Sec}$ molecule. A 'non-catalytic' dimer employs its N-terminal domain to dock the tRNA to the enzyme, whereas residues of the C-terminal domain of the 'catalytic' dimer orient the aminoacyl group into the active site for catalysis to occur. Herein, the mechanisms and structures of selenocysteine synthases are summarized.

Keywords PLP • SelA • Selenocysteine • Selenocysteine synthase • SepSecS • $tRNA^{Sec}$

9.1 Introduction

Selenium is the only essential dietary micronutrient that is found as a constitutive component in specific proteins and enzymes in all domains of life. Its precise location in selenoproteins is encoded in the DNA. The major physiological form through which selenium exerts its biological roles is selenocysteine, the 21st proteinogenic

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amino acid. Since its discovery in proteins [1–3], questions were raised about the importance and function of selenocysteine, and whether it could be substituted by isosteric amino acids, cysteine and serine. A typical selenoproteome is of limited size [4–6], but its members are important for the health, development, and organism survival. Although small in size [4], the human selenoproteome is vital for the maintenance of the cellular redox potential, protection of the membrane and DNA from oxidative damage, removal of reactive oxygen species, and regulation of the thyroid hormone homeostasis and metabolic rate (reviewed in [7–9]). Moreover, substitution of selenocysteine with cysteine or serine either completely diminishes or significantly reduces catalytic prowess of selenoenzymes [10–18]. Given that mutations affecting selenoprotein gene translation cause various pathologies including cancer (reviewed in [7–9, 19–23]) and that the mouse $tRNA^{Sec}$ knockout mutant is embryonically lethal [24], it is reasonable to suggest that the efficient synthesis and insertion of selenocysteine are fundamental biological processes. It is thus not surprising that significant efforts were invested in determining identities of macromolecular components, the sequence of events, and the mechanism(s) of discrete steps governing selenocysteine synthesis across kingdoms.

Early studies indicated that selenium is an essential component of archaeal hydrogenase, bacterial glycine reductase [3], nicotinic acid hydroxylase [25], formate dehydrogenase [1], xanthine dehydrogenase [26], and avian and mammalian glutathione peroxidases [27–29]. The assumption was that selenium was incorporated into proteins by an unknown post-translational mechanism. However, surprising observations that an in-frame UGA (*opal*) stop codon in the bacterial formate dehydrogenase and mouse glutathione peroxidase signals insertion of selenocysteine [30, 31], ignited an avalanche of extraordinary and unforeseen discoveries. Genetic and biochemical studies in bacteria have identified *selA*, *selB*, *selC*, and *selD* as essential genes for selenoprotein synthesis. The monocistronic *selC* gene [32] was shown to encode $tRNA^{Sec}$, which is serylated by the cytosolic SerRS, 'reads' the UGA codon, and supports insertion of selenocysteine with the aid of specialized elongation factor SelB [33]. $tRNA^{Sec}$ was subsequently shown to adopt a novel fold, 8/5 in prokaryotes and 9/4 in eukaryotes, characterized by 13 base pairs in the acceptor-TΨC arm, and extended D- and variable arms [34–38]. This is in contrast to a canonical 7/5 fold and a 12-base pairs long acceptor-TΨC arm found in all other elongator tRNAs. These results validated a proposition that selenocysteine is synthesized from serine [39] on its tRNA [40] in a reaction that requires SelD [40] and SelA [32, 41–44]. Concurrent studies on the bacterial and eukaryotic orthologs have established that SelD is selenophosphate synthetase, the enzyme that converts selenide into selenophosphate in the presence of ATP [45, 46]. On the other hand, it was suggested that SelA is a selenocysteine synthase that promotes conversion of Ser-$tRNA^{Sec}$ into Sec-$tRNA^{Sec}$ [41, 43]. Hence, a novel two-step indirect aminoacylation pathway for selenocysteine synthesis was proposed. In the first step, SerRS attaches L-serine onto $tRNA^{Sec}$ at the expense of one ATP molecule, and in the second, SelA promotes the serine-to-selenocysteine conversion while utilizing selenophosphate [41, 45].

Whereas the bacterial cycle of selenocysteine was delineated in the early 1990s, understanding of the analogous process in archaea and eukaryotes was significantly lagging. It took almost 20 years to ascertain that an unusual phosphoserine tRNA from mammalian brain, liver [47, 48] mammary glands [49], and avian liver [48], and the bovine liver *opal* suppressor serine tRNA [50, 51] represent, in fact, eukaryotic $tRNA^{Sec}$ [52]. After purification of a specific kinase that phosphorylates the *opal* suppressor $Ser\text{-}tRNA^{Sec}$ [53] it was proposed that the eukaryotic $Sec\text{-}tRNA^{Sec}$ is synthesized from $Ser\text{-}tRNA^{Sec}$ *via* a $Sep\text{-}tRNA^{Sec}$ intermediate [54–56]. This proposal was thereafter reversed and it was suggested that the eukaryotic and bacterial processes follow the same reaction mechanism [57, 58]. Around the same time, a report that autoantibodies from patients suffering from autoimmune hepatitis [59–61] precipitated a stable complex between a 48-kDa protein and $tRNA^{Sec}$ went almost unnoticed because the precipitated protein factor was considered to be involved in co-translational incorporation of selenocysteine [62]. The protein factor, known as soluble liver and pancreas antigen (SLA/LP) [59–61], was later sequenced, cloned and purified [63, 64]. The bioinformatics analyses have revealed that SLA/LP is well conserved in archaeal and eukaryotic organisms that contained selenoproteins and it is likely a PLP-dependent enzyme [65, 66]. Carlson *et al.* then identified murine *O*-phosphoseryl-$tRNA^{Sec}$ kinase (PSTK), which promotes the phosphoryl group transfer from ATP onto $Ser\text{-}tRNA^{Sec}$ and not onto $Ser\text{-}tRNA^{Ser}$ [67]. The discovery of PSTK not only lent credence to the original proposal that synthesis of selenocysteine in higher organisms proceeds *via* the phosphoseryl intermediate, but it also explained why phosphoserine tRNA and the kinase activity were detected in the first place [47–49]. In spite of this, the evidence that a specific enzyme can form selenocysteine from the tRNA carrying phosphoserine was missing. That such enzymatic activity may exist was suggested by an unrelated study on the tRNA-dependent synthesis of cysteine in certain archaea. Sauerwald *et al*. identified an archaeal enzyme, SepCysS, which converted $Sep\text{-}tRNA^{Cys}$ into $Cys\text{-}tRNA^{Cys}$ [68]. The enzyme was the Fold Type I PLP dependent enzyme, just like SelA. This prompted an intense search for an enzyme that could utilize $Sep\text{-}tRNA^{Sec}$ as a substrate. Two research groups independently identified murine and archaeal *O*-phosphoseryl-$tRNA^{Sec}$:selenocysteinyl-$tRNA^{Sec}$ (SepSecS; SepS) that supported selenocysteine and selenoprotein synthesis [69, 70]. The enzyme promoted phosphoseryl-to-selenocysteinyl conversion in the presence of selenophosphate while retaining stringent specificity towards $Sep\text{-}tRNA^{Sec}$. Importantly, murine and archaeal SepSecS were shown to be homologs of human SLA/LP identified in the early 1990s, which, in turn, explained why SLA/LP formed a stable complex with $Sep\text{-}tRNA^{Sec}$. Thus, all results suggested that the selenocysteine cycle in higher organisms is composed of three reactions. As in bacteria, SerRS first attaches serine onto $tRNA^{Sec}$. Subsequently, PSTK converts $Ser\text{-}tRNA^{Sec}$ into $Sep\text{-}tRNA^{Sec}$, and in the terminal reaction, SepSecS substitutes selenol for phosphoryl yielding $Sec\text{-}tRNA^{Sec}$. Although yielding the same reaction product, SelA and SepSecS use different tRNA-based substrates, implying that their catalytic mechanisms and structures are not completely conserved. In this chapter, the current understanding of selenocysteine synthases is presented.

9.2 The Structure and Architecture of the Bacterial SelA

Following genetic and biochemical studies that identified SelA as the bacterial selenocysteine synthase, significant strides were made to determine the structure and architecture of both holo SelA and the SelA:tRNA^{Sec} binary complex. The CryoEM studies on *Escherichia coli* and *Moorella thermoacetica* SelA established that ten enzyme subunits, each containing one PLP molecule, arrange into a ~500-kDa five-fold symmetric structure [71, 72]. However, the exact stoichiometry of the SelA:tRNA^{Sec} complex remained controversial [72, 73]. Most recently, Itoh *et al.* provided a wealth of structural and biochemical information on this system by studying *Aquifex aeolicus* SelA and *Thermoanaerobacter tencongensis* tRNA^{Sec} [35, 74]. The crystal structure of holo SelA revealed that the enzyme is a homo-decamer in which ten subunits are arranged into a pentamer of dimers (Fig. 9.1). Each SelA subunit is composed of three domains: an N-terminal domain (NTD; residues 1-66), a core domain (residues 90-338) and a C-terminal domain (CTD; residues 339-452), which fold into a structure typical of Fold Type I PLP-dependent enzymes (Fig. 9.1a). A short linker (residues 67-89) connects NTD with the core domain. The enzyme belongs to a distinct taxon that is likely to form an independent group related to the cystathionine γ-synthase family [74]. Structurally, two SelA monomers form an intimate dimer at which interface two active sites are formed. Each active site contains PLP covalently attached to a conserved Lys^{285}

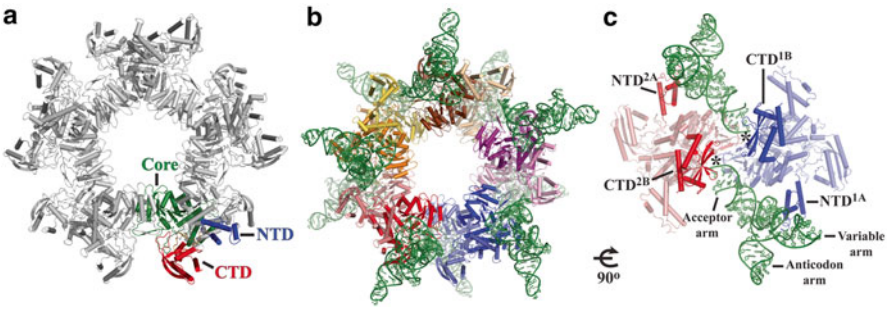

Fig. 9.1 Bacterial SelA forms a ring structure that binds ten tRNA^{Sec} molecules. (**a**) A ribbon diagram of holo SelA (PDBID: 3W1J), viewed above the ring plane, reveals a pentameric ring-like structure. Domains of one SelA monomer are colored: NTD is *blue*, the core domain is *green*, and CTD is *red*; other SelA subunits are *grey*. (**b**) The SelA:tRNA^{Sec} complex (PDBID: 3W1K) shows that ten tRNAs bind to the SelA decamer. tRNA is *green*, and SelA dimers are *red* and *pink*, *orange* and *olive*, *brown* and *beige*, *purple* and *light purple*, and *blue* and *light blue*. (**c**) Two intimate dimers (*pink* and *light blue*) bind two tRNAs. NTD of monomer 1 (NTD1A; *blue*) binds to the D-arm and T loop, and CTD of monomer 2 (CTD2B; *red*) interacts with the CCA-end. This is repeated on the other side of the dimer where NTD2A (*red*) binds to the D-arm and T loop of the second tRNA, and the acceptor arm binds to CTD1B (*blue*). *Asterisks* designate locations of the catalytic grooves

residue (see Sect. 9.4). The mobile NTD protrudes from the plane of the ring structure and interacts with the core domain of the neighboring intimate dimer (Fig. 9.1). On the other hand, CTD is oriented differently from that in other Fold Type I PLP enzymes, and this presumably enables formation of the pentameric SelA ring. The ring harbors large interdimer clefts that play an important function in tRNA binding and catalysis.

The crystal structure of the SelA:$tRNA^{Sec}$ complex provided the first hints at how SelA recognizes $tRNA^{Sec}$ (Fig. 9.1b). The binary complex crystal contained ten $tRNA^{Sec}$ molecules bound to the SelA decamer, thus suggesting 1:1 stoichiometry. $tRNA^{Sec}$ interacts with the enzyme through its extended D- and acceptor-TΨC arms, whereas the variable and anticodon arms do not participate in binding (Fig. 9.1c). Two intimate dimers bind one tRNA molecule in an arrangement in which one SelA dimer (e.g., subunits A, B or $SelA^{AB}$) binds the tRNA body, while the other one (e.g., subunits C, D or $SelA^{CD}$) positions and orients the aminoacylated CCA-end into the catalytic pocket (Fig. 9.1c). This observation implies that only in a decameric arrangement SelA forms a productive complex with $tRNA^{Sec}$. Indeed, mutations that disrupted the decamer structure inhibited the SelA activity, but retained ~90 % of its tRNA-binding capacity [74].

Perhaps the most important structural element for tRNA recognition is NTD of SelA, which binds the D-arm and TΨC loop of $tRNA^{Sec}$. The deletion of NTD abolishes binding of SelA to both Ser-$tRNA^{Sec}$ and unacylated $tRNA^{Sec}$ [35]. Moreover, this segment is needed to distinguish $tRNA^{Sec}$ from other tRNAs including homologous $tRNA^{Ser}$. Particularly, the NTD interacts with the fifth and sixth base pairs G14:U21 and C15:G20a of the D-arm, and with the triple base pair in the D loop. Because these base pairs are present in $tRNA^{Sec}$ only, it is postulated that they may be specificity elements for all enzymes involved in selenocysteine synthesis. The length of the acceptor-TΨC arm of $tRNA^{Sec}$ (eight nucleotides *vs*. seven nucleotides found in canonical tRNAs), which is important for binding the $tRNA^{Sec}$-specific elongation factor, is not critical for recognition by SelA due to conformational mobility of the NTD. Further, an intimate dimer that binds to the D-arm and T loop does not interact with the CCA-end and thus does not act on that particular $tRNA^{Sec}$ (Fig. 9.1c). Instead, this dimer positions $tRNA^{Sec}$ so that the acceptor arm can interact with the C-terminal domain of the neighboring intimate dimer. The tip of the acceptor arm binds to a large interdimer cleft where residues 423 and 424 of SelA interact with the first base pair G1:C72 and G73, the discriminator base. Mutational analyses have confirmed that recognition of the acceptor arm tip is important for selenocysteine synthesis [35]. Furthermore, structural modeling suggested that the binding pocket for A76 is composed of residues from subunits A and C, Asn^{218A} and Phe^{224C}, which are in proximity only in the pentameric SelA. Hence, a model of tRNA recognition by SelA emerged in which one SelA dimer deploys NTD to dock $tRNA^{Sec}$ onto the decamer, while the neighboring dimer uses CTD to interact and position the seryl-CCA into its catalytic groove. This pattern repeats on both sides of the SelA homodecamer yielding a large ribonucleoprotein assembly capable of simultaneously acting on ten tRNA substrates.

9.3 The Structure of the Archaeal and Eukaryotic SepSecS

The X-ray crystallography studies on the archaeal and murine holo SepSecS [75, 76], and the human SepSecS:tRNA^{Sec} binary complex [37] revealed the structure of selenocysteine synthase in higher organisms. SepSecS is a tetramer composed of a dimer of intimate dimers (Fig. 9.2a). The intimate dimer interface encloses two active sites each carrying one PLP molecule covalently linked to a conserved Lys^{284} (see Sect. 9.4). SepSecS is composed of three domains: an extended NTD (residues 1–130), a core domain (residues 131–315), and a CTD (residues 360-501) (Fig. 9.2a). A linker composed of long α-helices (residues 316–359) joins the core domain and CTD. With the exception of the extreme C-terminus, the overall structure of SepSecS is well conserved. The CTD of the archaeal SepSecS is shorter than the mammalian counterpart; it lacks a segment (residues 473–493) identified as the antigenic region in patients suffering from chronic autoimmune hepatitis (reviewed in [20]).

SepSecS forms its own branch in the phylogenetic tree of Fold Type I PLP-dependent enzymes that may be related to the sugar aminotransferase family [74, 76]. SepSecS is a primordial enzyme, probably present in the last common ancestor. A certain level of structural homology with the dimeric SepCysS and selenocysteine lyase is evident in the core and C-terminal domains. However, both of these homologs lack the NTD, an element pivotal for oligomerization of SepSecS. In particular, N-terminal helices α1, α2, and α4 from each subunit interact with one another and establish a hydrophobic core of the tetramer. The importance of these interactions

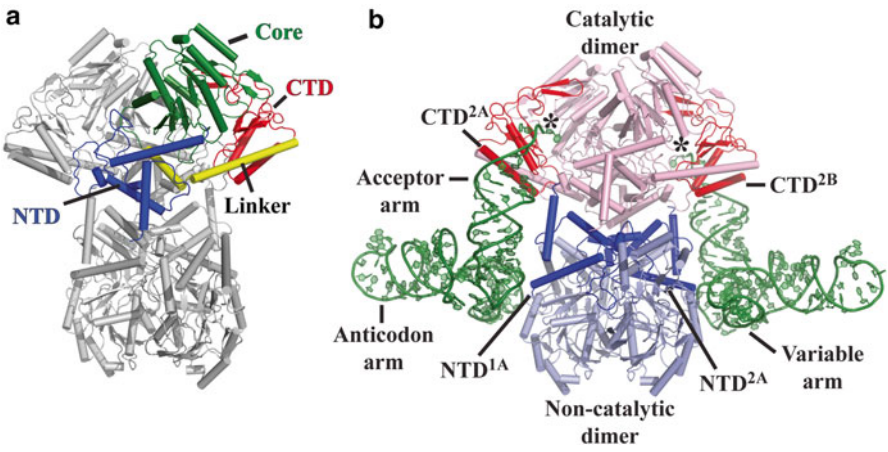

Fig. 9.2 SepSecS homotetramer binds up to two tRNA^{Sec} molecules. (**a**) Two SepSecS homodimers arrange into a tetramer. Domains of one subunit are colored: NTD is *blue*, the core domain is *green*, linker is *yellow*, and CTD is *red*. The rest of the tetramer is *grey*. (b) The binary complex structure (PDBID: 3HL2) shows that NTD (NTD1A, NTD2A) of the non-catalytic dimer (*light blue*) binds to the acceptor arm of tRNA, and that CTD (CTD2A, CTD2B) of the catalytic dimer (*pink*) binds to the tip of the acceptor arm. *Asterisks* mark the catalytic grooves (see Fig. 9.3 for more detail)

for tetramerization was shown by studies in which the deletion of helix α1 yielded a dimeric enzyme incapable of supporting selenocysteine synthesis [76]. Thus, the appended NTD of SepSecS and the uniquely oriented CTD of SelA [35] are the major oligomerization elements in selenocysteine synthases.

The crystal structure of SepSecS:tRNA^{Sec} revealed the architecture of the binary complex, the mechanism of tRNA recognition, and provided hints about the complex stoichiometry and reaction mechanism [37]. The structure showed that two tRNA^{Sec} molecules are bound to the SepSecS tetramer in a cross-dimer fashion (Fig. 9.2b), suggesting that only the tetrameric SepSecS can bind and presumably act on Sep-tRNA^{Sec}. However, SepSecS binds to the opposite side of tRNA when compared to SelA. The N-terminal helix α1 of the non-catalytic dimer binds to the long acceptor-TΨC arm, which is the major recognition element, and a segment of its core domain interacts with the long variable arm [37]. The tip of the arm binds to the C-terminal helices α14 and α15 of the catalytic dimer. Three conserved arginine residues in helices α14 (Arg^{398}) and α15 (Arg^{453}, Arg^{456}) interact with the G1:C72 base pair and the G73 discriminator nucleotide [37]. Recognition of the G73 discriminator is important for selenocysteine synthesis [37]. In addition, while there is no evidence that SepSecS undergoes a conformational change upon tRNA binding, the superimpositioning of the complexed [37] and unbound tRNA^{Sec}[38] revealed that the acceptor-, TΨC- and variable arms rotate around the vertical axis projecting through the anticodon arm upon complex formation [77]. This may be pivotal for orienting the phosphoseryl-CCA towards the catalytic groove.

Although SepSecS harbors four tRNA-binding sites, the binary complex crystal contained only two tRNAs bound to the tetramer. The same complex stoichiometry was observed when mimics of Sep-tRNA^{Sec} were used in binding assays [78]. The results of fluorescence-quenching binding assays and small angle X-ray scattering analyses confirmed that SepSecS preferentially binds either one or two tRNA^{Sec} molecules at a time [79]. This suggests that SepSecS employs a half-sites activity, but it remains to be seen if this functional asymmetry is of physiological significance and if it is regulated.

9.4 Divergent Active Sites of Selenocysteine Synthases and a Conserved Catalytic Mechanism

Both SelA and SepSecS harbor active sites formed at the interface of intimate dimers. Before atomic resolution structures were available, detailed enzymatic studies on SelA and SepSecS were pursued with the aim to delineate the possible catalytic mechanism. The SelA-catalyzed substitution of selenol for hydroxyl proceeds through an anhydroalanyl intermediate covalently attached to PLP [41]. In presence of SelD, reduced selenium, ATP and sodium borohydride, SelA converted Ser-tRNA^{Sec} into Ala-tRNA^{Sec} with a fraction of pyruvate being released into solution. The reaction was stoichiometric with 2 mol of enzyme acting on 1 mol of

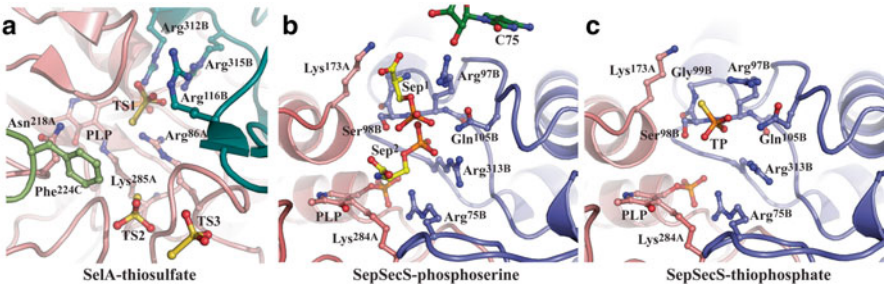

Fig. 9.3 Catalytic sites in SelA and SepSecS and binding pockets for phosphoserine and selenophosphate. (**a**) The active site in SelA is composed of residues from subunits A (*teal*) and B (*pink*) of the intimate dimer, and at least one residue from the neighboring dimer subunit C. Thiosulfate ion TS1 (*gold sticks*) designates the binding pocket for selenophosphate, and TS2 and TS3 mark the A76-binding pocket. (**b**) The active site in SepSecS is composed of residues only from subunits of the intimate dimer (*pink* and *blue*). Free phosphoserine (Sep1, Sep2; *gold sticks*) binds in two orientations, none of which are optimal for catalysis. (**c**) Thiophosphate (TP; *gold sticks*) binds to the P loop (Ser98-Gln105), like TS1 in SelA. Catalytic residues in SelA (**a**) and SepSecS (**b**) are shown as sticks

Ser-tRNA^{Sec}. Studies on the archaeal [69], murine [70] and human [37] systems confirmed that PLP is required for phosphoserine-to-selenocysteine conversion, but the anhydroalanyl intermediate remained elusive. This discrepancy was ascribed to the instability of reaction intermediates bound to SepSecS. Recent structural and mutational studies provided a wealth of information about the active sites of SelA and SepSecS, and possible catalytic mechanisms.

The crystal structures of SelA in complex with thiosulfate (TS) [35], holo SepSecS [75, 76] and the human SepSecS:tRNA^{Sec} complex [37] revealed the three-dimensional structure of the active sites and suggested residues pivotal for catalysis. In the SelA structure, TS1 binds to the selenophosphate-binding pocket composed of Arg86A, Arg312B and Arg315B (Fig. 9.3a). The pocket sits atop PLP, which is coupled *via* Schiff base to Lys285A. When a string of arginine side chains was mutated into alanines, the catalytic activity of SelA was markedly reduced. The side chains of Arg119B and Asp284A, which interact with Arg86A and Arg312B, might be important for catalysis (Fig. 9.3a). Intriguingly, TS2 and TS3 bind near Asn218A and Phe224J, which are spatially proximal to the TS1-binding pocket. Because mutations of these two residues significantly diminished SelA activity, it was proposed that TS2 and TS3 mimic the binding of A76. As a corollary, this proposal suggested that only as a pentameric ring could SelA bind Ser-tRNA^{Sec}, which was later shown to be correct [74].

Further, the crystal structures of SepSecS provided a comparable level of structural information. We shall briefly discuss only the human enzyme, but with a note that the same conclusions most likely apply to the archaeal and other eukaryotic orthologs. The SepSecS subunit of the catalytic dimer that interacts with the CCA-end (monomer A) provides PLP linked to Lys284A, Gln172A, and Lys173A (Fig. 9.3b). The other subunit (monomer B) provides the P-loop (Ser98B and

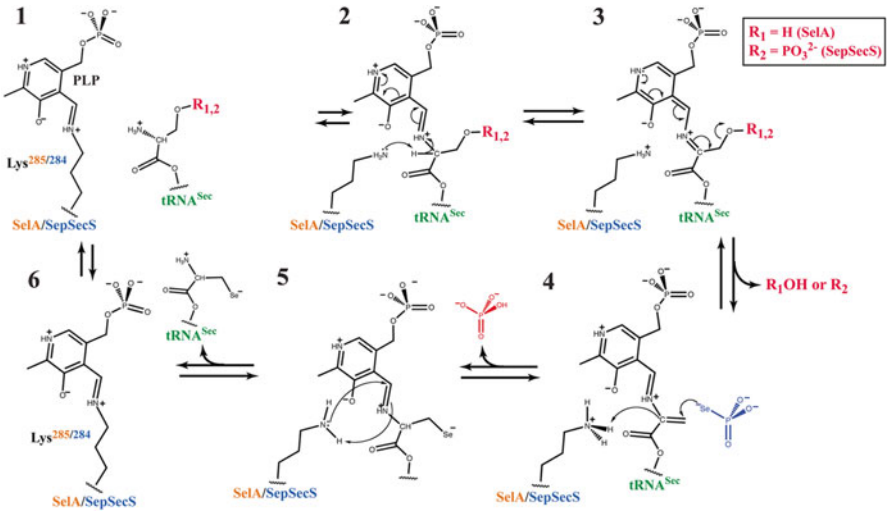

Fig. 9.4 Proposed PLP-dependent mechanism of the terminal reaction of selenocysteine synthesis catalyzed by SelA and SepSecS. For details on the mechanism see Sect. 9.4 of the main text and Fig. 4 in Refs. [37] and [35]. The leaving groups in aminoacyl groups are highlighted in *red*: R_1 (SelA) is H and R_2 (SepSecS) is PO_3^{2-}

Arg97B), which binds thiophosphate, and Arg75B, Gln105B, and Arg313B that are important for catalysis (Fig. 9.3b, c). Free phosphoserine binds to the active site of the catalytic dimer only, but in two orientations neither of which are optimal for catalysis. The covalent attachment of phosphoserine to $tRNA^{Sec}$ is thus essential for its proper placement into the active site. Because thiophosphate binds to the similar site in the non-catalytic dimer, it was suggested that selenophosphate binds to SepSecS only after phosphate is eliminated from Sep-$tRNA^{Sec}$.

Taken together, a unified PLP-dependent mechanism for the terminal reaction of selenocysteine synthesis was proposed (Fig. 9.4) [37, 41, 75, 76]. The reaction begins with binding of Ser-$tRNA^{Sec}$ and Sep-$tRNA^{Sec}$ to SelA and SepSecS, respectively. The amino group of the seryl/phosphoseryl moiety attacks the Schiff base thus yielding the external aldimine. The liberated Lys285/284 side chain abstracts the Cα proton from serine/phosphoserine. The electron delocalization by the pyridine ring leads to β-elimination of water and phosphate from Ser- and Sep-$tRNA^{Sec}$, respectively, and the anhydroalanyl-$tRNA^{Sec}$ intermediate is formed. Upon water/phosphate release, selenophosphate binds to the P-loop. The concomitant attack of water on selenophosphate and of the nucleophilic selenium onto the anhydroalanyl moiety yields an oxidized form of Sec-$tRNA^{Sec}$ while releasing a second phosphate equivalent. Alternatively, selenophosphate attacks anhydroalanine and forms phosphoselenyl-$tRNA^{Sec}$, which subsequently breaks down to Sec-$tRNA^{Sec}$ after water attack. Lastly, Lys284/285 re-establishes the internal aldimine, and Sec-$tRNA^{Sec}$ is released from the enzyme. It is suggested that, besides PLP, non-homologous arginine residues play a critical role in catalysis. However, further structural and

enzymatic studies are needed to both define precise role(s) for these residues and provide a complete picture about the remarkable mechanism of selenocysteine synthases.

9.5 Future Directions

Despite the remarkable progress, important questions about selenocysteine synthases and their biological roles remain unanswered. In particular, SelA and SepSecS were crystallized with the unacylated $tRNA^{Sec}$ and interactions of the seryl and phosphoseryl groups with the active sites were not visualized. Also, anhydroalanyl-$tRNA^{Sec}$ bound to SepSecS has not been captured, leaving the exact mechanism in the dark. Given that SelA and SepSecS are downstream of SelD and SPS2, it would be important to assess if they regulate the overall selenium homeostasis. Furthermore, it would be important to establish if the half-sites activity of SepSecS is allosterically regulated. Because SelA and SepSecS bind $tRNA^{Sec}$ from the opposite sides when compared to SerRS and PSTK, respectively, it is plausible that multi-enzyme 'selenosomes' facilitate selenocysteine synthesis. Lastly, given recent clinical reports [80–83], studies on the role of SepSecS and selenoproteins in the development and maintenance of the healthy human brain are warranted.

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